

SALT can have too high a price

WITH the last details settled in the second round of Strategic Arms Limitation Talks (SALT-2), a treaty which has been under negotiation for more than four years is closer to reality. The agreement will be signed by the heads of state of the two superpowers in a month's time, but on the United States' side there will still remain one major hurdle—ratification by the US Senate with at least 67 of 100 votes in favour. And there are all manner of reasons why 34 senators could be persuaded to vote against ratification.

The fundamental principles of the treaty have been well-known for some time. Each side's 'strategic' launchers will be limited in number to 2,250. This figure is slightly higher than the present US total of launchers, including inter-continental ballistic missiles (ICBMs), heavy bombers and submarine missile tubes. It is 250 lower than the Soviet total, so there will have to be some dismantling on the Soviet side. There will be no specific restriction on the weight these launchers may carry, but not more than 820 ICBMs may be fitted with multiple independently targetable re-entry vehicles. In a first grapple with the problem of low altitude cruise missiles their range will be restricted to 600 kilometres.

Rhetoric on the need for SALT 2 is predictably hotting up. At various times President Carter has claimed that it will "make the world safer and more secure", that ratification will be "the most important single achievement that could possibly take place for our nation during my [Carter's] lifetime" and that failure to ratify would release nuclear weapons constraints on countries such as Pakistan, India, South Korea, Taiwan and South Africa. But despite these questionable assertions and despite an apparent general support for SALT-2 in the US, there are still identifiable groups opposed to ratification.

There are, not surprisingly, those whose political complexion does not predispose them to favour any deal at all with the Soviet Union. Then there are those who would only favour a deal with the Soviet Union if it were coupled with other issues such as human rights and military constraints elsewhere (such as Africa). Further, some, not in principle opposed to arms-control agreements, will find SALT-2 in one way or another not an adequate measure. And there will be those who have great concern that the

US procedures for verification, despite firm assertions to the contrary, do not guarantee that the Soviet Union cannot cheat without certainty of detection.

There is, however, another position from which one can have the deepest concern about SALT-2 whilst holding the most impeccable credentials in favour of arms control. It runs as follows.

In the process of 'selling' SALT-2 to Senate, President Carter is going to have to reassure many of the waverers that he is not dismantling US strength in the strategic field. He has already said that the treaty will allow the US to pursue "all the defence programmes we believe we may eventually need", and this list is formidable. At the top of it undoubtedly come a new mobile missile (the MX) and the Trident submarine with its associated missiles. But there are also new air-, ground- and sea-launched missiles, a new bomber, and aircraft to carry cruise missiles. This year's defence budget also shows that there will be more spent on basic and applied research; favoured areas in future will be space (particularly the Shuttle), lasers and anti-satellite measures. The phrase of the particle physicists "all that isn't forbidden is compulsory" is easily transferred to the world of military procurement.

It does seem that the price that will be exacted for SALT-2 is going to be commitment to a disturbingly wide range of new military programmes. Those with long memories will recall that in 1963 a somewhat similar situation arose when the price for the Partial Test Ban was a commitment by President Kennedy to vigorous underground weapons testing, which effectively degraded an arms control measure into an anti pollution measure.

Some thoughtful people will wonder whether this sort of game is worth the candle. If the only way to achieve arms control is to turn up the volume elsewhere, isn't it time to stop deluding ourselves that the SALT agreements are so valuable? On balance such sentiments should not lead to a vote against ratification, because if ratification fails the forces of aggressive military procurement succeed even more strongly. But there certainly should be pause for thought, and SALT-3, bringing in European nations, should not be yet another occasion for simultaneously slowing and speeding up the arms race. □



NASA carpeted over Space Shuttle costs

THE US National Aeronautics and Space Administration, deeply embroiled in technical problems over the final stages of the Space Shuttle development programme, is facing what some employees are already referring to as the worst setback to have hit the agency since the launch-pad fire in the Apollo programme. Last Friday, President Carter asked the US Congress for a budget amendment that would increase NASA's budget for 1980 by \$220 million over the present request of \$4.6 billion to absorb the extra costs of the Shuttle programme (the need to accommodate which had already led to the exclusion of any new starts in the 1980 budget).

The request for the extra money followed the revelation two weeks ago by Dr Robert Frosch, administrator of NASA, that the agency was now expecting cost over-runs due to last minute delays in the Shuttle programme to exceed \$600 million over the next four years. This figure is considerably higher than estimates provided earlier this year to Congressional committees in their discussion of NASA's 1980 request, during which agency officials gave little indication that the projected budget would be insufficient.

In a sharply worded letter sent to Dr

Frosch last week, Senators Howard Cannon, Adlai Stevenson Jr and Jack Schmitt, of the Senate Committee on Commerce, Science and Transportation, say that the failure to alert the committee about the critical funding situation was "most regrettable". "The current situation seriously threatens that committee's credibility as well as NASA's. This condition must be rectified promptly", wrote the three members of the Committee, which is responsible for recommending the agency's budget to the Senate.

The three committee members say that a thorough review and identification of specific failures, together with a statement of corrective action initiated or proposed, is necessary. "Coupled with support for the optimum solution, which will be reflected in the bill the committee recommends to the Senate, is the need for a forthright explanation of why we are confronted with the present situation and concrete assurance that management deficiencies are corrected."

Dr Frosch told the committee members at the end of last week that NASA has already established an inquiry, under the direction of Dr Alan M. Lovelace, deputy administrator of the agency, to look into the causes of the current production delays and cost

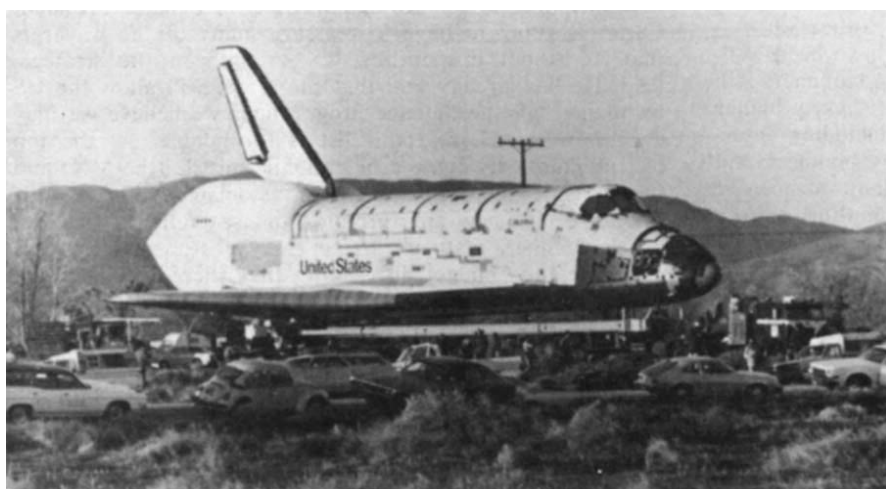
over-runs. Within the agency there are already rumours that several heads might fall as a result of the inquiry.

The main technical problem facing the Shuttle, whose first flight is officially still scheduled for November but now seems unlikely to take place before the end of the year, is at present the task of fixing the heat-resistant tiles to the surface of the re-entry vehicle. Although the tiles were meant to be fixed at a current rate of about 500 a week, problems in handling and fixing the tiles to the surface of the vehicle have meant that progress is nowhere near that figure.

At present NASA hopes that with the increased funding that it is requesting (in addition to the budget amendment, the agency has also put in for an extra \$185 on top of its 1979 budget), as well as a rescheduling of some of the early flight plans, the delays will have a minimal impact on any of the planned space science operations. But in a time of general financial stringency, there have already been moves in the House of Representatives to reduce Space Shuttle funding to provide more money for mass transportation. And although these have so far been unsuccessful, if Congress refuses to grant the extra money, a number of projects could be in peril.

Already representative Edward P. Boland, chairman of the House Appropriations subcommittee responsible for NASA's budget, has said that he believes money could be taken out of both the Space Telescope and the Galileo Jupiter mission to help cover some of the additional Shuttle costs. Officials with the European Space Agency are concerned that any delay in the Space-lab launch, currently scheduled for August 1981, could add considerable additional costs to a project which has already over-run estimates by 40%.

Precise details of NASA's plans are likely to emerge in new hearings which the Senate Science and Space Subcommittee, chaired by Senator Stevenson, is planning to hold on the budget amendment request. **David Dickson**



Fixing the heat resisting tiles is proving difficult . . .

US signs treaties on scientific links with China and Japan

Mrs Juanita Kreps, US secretary of commerce, and deputy Chinese prime minister Fang Yi, last week signed four agreements on scientific and technological cooperation in fields ranging from weather monitoring to tuna fishing.

The agreements allow for Chinese scientists to work on measurements and standards at the Bureau of Standards in Maryland, and at the

Severe Storm Center in Oklahoma. US scientists will visit Peking, where the agreements were signed, to computerise the Chinese weather-forecasting system, and to train Chinese in the use of the US technology which they are planning to import.

Exactly one week earlier, the US and Japanese governments signed an agreement in Washington on co-operation in research and development

in energy and related fields, the outcome of a Japanese initiative to President Carter last May.

Areas covered by the latter agreement include fusion research—with the Japanese planning to participate in the Doublet III magnetic fusion project—coal conversion, solar energy conversion through photosynthesis, geothermal energy, and high energy physics. □

Kennedy attacks Carter plan to cut biomedical research



SENATOR Edward Kennedy (above) last week criticised as "surprising and disappointing" President Carter's budget recommendation that funds for biomedical research should be kept constant between 1979 and 1980. Allowing for inflation, this would mean a 10% cut on research spending.

The senator's remarks were made in an address to a group of medical research societies, including the American Federation for Clinical Research and the American Society for Clinical Investigation. His comments were welcomed by the 2,000 scientists present, as was his promise to take steps to reduce the amount of paperwork research workers are now required to complete. However, other suggestions included in a bill which Senator Kennedy has introduced into the Senate, such as a proposal to establish a President's council to prepare five-year plans for medical research, and the experimental inclusion of lay-people on some peer review committees, are being less warmly greeted by the medical community.

In his speech last week, Senator Kennedy, who is chairman of the Senate sub-committee on health and science, said that the Administration's budget proposal to reduce spending on biomedical research next year—which Administration officials defend by pointing to the 25% increase in the current year—was both short-sighted and regrettable. "In 1967, the United States invested 4.9% of its health dollars in health science research. Today that figure stands at 3.4%, and if the current Administration policy stands, that percentage will plummet further still," he said. Keeping up with inflation was the minimum that should be done to fund health science programmes.

But money alone was insufficient; visits to research centres around the country had convinced him that:

● It was time for Congress to commit

itself "openly and squarely" to the support of investigator-initiated research in both the fundamental and the clinical sciences.

● Health science research workers were "drowning in paperwork and reporting requirements". Some scientists were spending as much as a quarter of their time filling out forms and doing mundane administrative chores.

● The Federal Government should restore some continuity and predictability to its federal health research budget, avoiding the recent series of confusing swings in research priorities at the federal level which were "unfair to scientists who have dedicated themselves in good faith to the directions mandated by the Federal Government."

Referring to the Health Science Promotion Act which he has recently introduced with Senator Javits to tackle some of these problems, Senator Kennedy asked the scientists for their help in making this "the kind of wise and constructive legislation which can help prepare our health science institutions for challenges of the 1980s". Some of the provisions in the proposed act, such as the legal status that it would provide to the National Institutes of Health (a status currently enjoyed only by its constituent institutes) have been generally welcomed by the medical research community, who recognise many of the symptoms that the senator described.

Other suggestions, however, have generated considerable controversy. These include the appointment by the President of an independent council to prepare and up-date a five-year forward look for health sciences, the involvement of scientists from other disciplines (such as physics or statistics) on NIH review panels; and to place, as an experiment, lay people on a sample number of peer review panels. Also controversial is a proposal that the mandates of the various institutes within the NIH should be renewed by Congress every few years, the first period for renewal coming up in 1983. "To me that is antithetical to any long-range planning mechanism", one NIH staff member said.

There is also criticism of Senator Kennedy's suggestion that the director of NIH should have to ensure that at least 45% of NIH's biomedical research funds go to support investigator-initiated research. At present, about 43% of funds are distributed this way, but NIH officials point out that things are never the same from one year to the next. □

Library report calls for a national periodicals centre

THE setting up of a 'national periodicals centre' to compensate for the increasing number and price of academic journals—particularly in scientific and technical subjects—has been recommended by the National Enquiry on Scholarly Communication. In a three-year, \$600,000 study of research libraries published in New York last week, the national enquiry says that further growth in the number of scholarly journals should be discouraged, and concludes that major research libraries cannot expect major budget increases over the next decade.

The report says that if this conclusion is valid, "research libraries can no longer function as autonomous entities". And given the growing resistance to interlibrary loan, it recommends a national periodicals centre to provide libraries with a reliable source of copies of articles from journals too seldom used to warrant library subscription.

"The creation of a national periodicals center will lead to a changing environment for journal publishers, particularly in the science and technical disciplines," the report says. On the one hand, librarians would have a new option to consider: on the other, "the capacity created in the periodicals centre should increase the market for alternative forms of publications" such as storage on microform.

Among the reasons for such a centre having a greater effect on scientific disciplines are, the report says, that:

● Subscription rates for scientific journals are far higher than they are for the humanities, so library savings from cancellations will be greater

● There is often a premium on speed of publication and access in scientific fields, and the periodicals centre and improved bibliographic service will increase the attraction of using new forms of publication

● The market for scientific and technical materials is much broader than for the humanities, since many commercial firms follow the scientific literature.

The report admits that some scholars and journal editors have expressed fears about the impact of a national periodicals centre. It adds, however, that "there is simply no substitute for browsing through the current journals as a stimulus to thought and further research, and librarians are as aware of that as are faculty members." □

Scholarly Communication is available from Johns Hopkins University Press, Baltimore 21218, US, price \$3.95.

From solar system to social system

David Dickson talks to John Gibbons (right), new director of the US Office of Technology Assessment

JOHN Gibbons is fond of quotations. He starts with one from Abraham Lincoln about the inadvisability of announcing future plans when one has only just entered office; ranges from Winston Churchill to Deep Throat's advice to "follow the money"; and uses Walt Whitman to support his interest as a scientist in the unity of nature. The ability to absorb the thoughts of others, and mould a set of eclectic facts and opinions into a meaningful synthesis that represents more than mere repetition, is probably one of the most important assets that he brings to his new job as director of the US Congress' Office of Technology Assessment.

"My past experience working with interdisciplinary teams taught me to listen very hard," he says of his experience as Director of Environmental Programs at Oak Ridge National Laboratory and, subsequently, the University of Tennessee.

Dr Gibbons, an approachable 50-year-old physicist, is the first academic scientist to be appointed to head the OTA, which was set up by Congress six years ago. He is also considerably less known in Washington political circles than his two predecessors, ex-congressman Emilio Q. Daddario, and more recently Dr Russell Peterson, an industrial chemist who was previously Governor of Delaware, and resigned from OTA two months ago.

After the political wrangles that have formed the back-drop to OTA's recent history, Dr Gibbons' appointment has (to the relief of many OTA staff) been mercifully swift. The question now is whether he can produce the peculiar blend of technical, managerial and political sensitivities that the job requires.

Despite a low public profile, he is no stranger to Washington. He was a director of the Federal Energy Administration's Office of Energy Conservation between 1973 and 1974 and he sits on numerous advisory boards on energy and environment related issues where he has built a reputation for both fairness and independence.

His professional roots, however, lie in the academic community. A graduate of Duke University, Dr Gibbons joined Oak Ridge in 1954, and spent fifteen years as a nuclear geophysicist, eventually heading a team investigating how experimental nuclear physics provided clues about the formation of the solar system (as well as the surface of materials).

Professional and vocational interests

combined in 1970 when he set up and directed an environment programme at Oak Ridge, studying in particular the relationship between energy and environmental issues, and developing techniques of energy demand analysis. This lead directly to considering the political process. "I recognised as I tried to wrestle with problems related to, for example, the efficient use of energy, that much depended on the confluence of good technical information into the policy process," he says.

Much of his approach is pragmatic. A report recently released by OTA on home energy conservation, for which Dr Gibbons chaired the advisory panel, concluded that home owners were more likely to adopt energy efficient practices by being convinced that they would save money than by appeals to sacrifice "which may be ineffective, if not counterproductive."

But he is also aware of the delicacy of OTA's role, suspended uneasily between the scientific and technical community on the one hand, and the political community on the other. "The problem is that OTA is expected to be objective and even-handed, but we also have to deliver information that is useful", he says. "Scientists and technologists can integrate over the technical aspects, but cannot handle the broader issues. OTA has to do a second-order integration, bringing in the economic and equity aspects. Congress then has to work out the political trade-offs."

He holds two paradigms (both from direct experience) of the way that OTA should proceed. The first was an intense, 30-day study of proposed plans for the Energy Research and Develop-



John Gibbons: listens very hard

ment Administration (now the Department of Energy), which he claims was largely responsible for getting Congress to include a conservation programme in the agency. The other is the residential conservation report, which took two years to prepare, but which shows in detail how conservation measures could generate energy savings of at least 13 billion barrels of oil by the year 2,000.

Both of these represented that type of structured interaction which he feels are essential if OTA's interdisciplinary efforts are to succeed. "Exxon designed a building in which you had to cross a library to get to your office. You need to plan and structure the process of social interaction if you are to get people to talk to each other. But this must be done through invisible organisation, rather than the heavy hand of management." His last quote is a favourite one from Adlai Stevenson: "Freedom rings when opinions clash." □

Soviet dissident charged

JUST a year to the day from the trial of Yurii Orlov, Vladimir Skvirskii, a Russian geologist and human rights activist, is due for trial this week, charged with "misappropriating state property"—library books.

This does not, it must be stressed, reflect a new Soviet tightening up on its literary holdings. Indeed, Skvirskii's defence counsel, contacted by telephone, said that he had never heard of anyone being put on trial before for failing to return a book, except for the occasional speculator who purloined and then sold books from libraries. In cases of negligence, the culprit simply had to pay a fine or offer another book in compensation.

This Skvirskii would willingly do. The books in question, he admits, were issued to him, but became damaged when he took them on a geographical expedition. The books, however, appear to have been only an excuse. During a police search of Skvirskii's home, using instruments including a mine-detector, some 69 objects were removed—nine library books and a large number of documents relating to the Soviet human rights and independent trade union movements. Witnesses questioned in the course of preliminary investigations were asked not about library books but about Skvirskii's political views, activities and contacts.

Vera Rich

Science journalists become activists

Science journalists must play an active role in development, and not be mere transmitters of technical information. This argument for a marked departure from the journalists' traditional 'neutral' role came not from an outside body, but from science journalists themselves, meeting last week in Laxenburg, near Vienna. The conference, part of the run-up to UNCTSD in Vienna in August, was organised by the European Union of Associations of Science Journalists.

There is a north/south imbalance in science and technology that is "increasing the gap between rich and poor", the journalists argue in their 'Laxenburg Declaration'. This is exacerbated by an information imbalance: "information is dominated by the industrialised countries".

The role of science journalists, then, is to help redress these imbalances. But they stressed that this is not simply a passive act—"the science journalist is a partner in development". In particular, one role of the science journalist is to "strengthen the bargaining position of developing nations in negotiations with suppliers of technology"—multinationals and foreign governments.

This new active posture carries risks. The declaration warns that "in the exercise of their role to aid development, science journalists may offend governments, media owners, and other powerful forces. In both the industrialised and non-industrialised world, they face punishment and jail". The journalists included the reference to industrialised nations in the latter sentence in recognition of the trial of science journalist Duncan Campbell in Britain last year.

Naturally, the journalists supported freedom of information and freedom of the press. But they pointed out that in the context of science this means that "free access to information in government and private research institutions should be guaranteed. Special attention should be given to the access to information from industrial and military research, in particular from companies and agencies operating on a world-wide basis".

Underlying the declaration was an acceptance of a view gaining increasing currency in the Third World—that the industrialised nations, through their aid policies and their multinational companies, are trying to impose particular patterns of technology on the developing world. It is up to the developing countries to decide which technologies are appropriate and, indeed, whether to adopt so-called appropriate technologies at all. □

Mössbauer's lab destroyed by fire

PROFESSOR Rudolf Mössbauer, Nobel prizewinner for physics in 1961, has lost his entire laboratory in an intense fire which raged in the Technical University of Munich in Garching last Saturday morning. According to first estimates, the fire began in the middle of an open corridor in the laboratory, setting light to a small theoretical physics library above. Arson is suspected, and the whole Garching site of the university has been closed off and is under strict security.

The entire building containing Mössbauer's laboratory was destroyed, taking with it a laser physics and low temperature laboratory of Professor Helmut Kinder.

Some of the equipment may be salvageable, but the damage has been aggravated by corrosion. The fabric of the building contained large amounts of polyvinylchloride, which with the heat of the fire and contact

with the water used to extinguish the blaze, produced hydrochloric acid. This has corroded equipment which was otherwise undamaged. Meanwhile the staff of other departments on the site are rallying round to offer room to the disestablished researchers. However it will take at least a year—and maybe several—to rebuild the laboratory to its previous condition, and the experiments that were in progress have been lost.

The extent of the damage has been estimated between 5 and 20 million DM. The equipment and laboratory were insured.

If the fire was the work of an arsonist—and that is by no means established—speculation will begin on possible motives. Professor Mössbauer is known to be a man of firm and vocal right wing views, and a political motive would not be entirely out of the question. □

Oil giants exaggerated Harrisburg-Izvestiya

"SLIGHT unpleasant consequences" was how academician Anatolii P. Aleksandrov, President of the Soviet Academy of Sciences, described the outcome of the Harrisburg nuclear accident.

Commenting in the paper *Izvestiya* on future world prospects for nuclear power, Aleksandrov maintained that the media coverage of Harrisburg had been "exceptionally over-stated". This, he implied, was due to the oil interests which "control the energy production of practically all the capitalist and many of the developing countries" and whose drive for maximum profits "is opposed to the interests both of the countries which possess petroleum, but also often to the interests of the petroleum consumer countries".

The Soviet Union has a major commitment to fast-breeder power generation, and the Western alarms which followed Harrisburg caused one notable change in policy. Until now, Soviet officials have been reluctant to admit to any accidents at their nuclear power stations. A few days after Aleksandrov's article, however, Peter Neporozhnii, who is the Soviet Minister of Power and Electrification, admitted to western journalists, that the Soviet Union had had a few nuclear mishaps. No details were given, but it was assumed that he referred first and foremost to the Shevchenko semi-pilot fast breeder, where in 1973 there was a "conventional" explosion when a ruptured heat exchanger brought hot sodium and water into contact.



Meanwhile Czechoslovakia continues to deny the two nuclear accidents which the Chartists allege took place in January 1967 and February 1977. They have, however, in the wake of Harrisburg, announced considerable improvements in their nuclear safety programme. Speaking on the English-language service of Prague radio, Z. Dlouhy, the head of the Radiation Safety Department of the Nuclear Research Institute at Rezh, said new legislation "places the country among the most advanced in the world in this respect". Prospective sites for power stations, he said, are evaluated from the point of view of numerous external effects, which range from average precipitation and wind patterns to seismic activity and the possibility of aircraft crashing on the plant. □

news in brief

Women scientists paid 20% less than men: Despite considerable growth in the number of women on US science faculties between 1973 and 1977, women's salaries still lag significantly behind those of men, according to a report presented to the Office of Science and Technology Policy by the National Research Council. The NRC says that the difference in salaries between the sexes was estimated to be about 20% overall in 1977 and remains a serious problem. The proportion of women faculty who received tenure also lags behind the proportion of men. At professorial level, the discrepancy between the salaries of men and women was at least \$2,500. The highest difference appeared in chemistry, where male professors were paid an average of \$6,200 more than female professors. The report recommends offering career development awards to help increase the number of women on science faculties. It also says that all public and private institutions should be required to include comparisons of salary and tenure in their official report.

AT out at UNCSTD: Appropriate technology and "basic needs", two of the areas stressed by development aid agencies, will be played down at UNCSTD, a high UN official said last week. However, the UNCSTD secretariat argues that since specific technologies are not on the agenda, nothing was to be heard of AT anyway. Both AT and "basic needs" are seen by the developing countries as paternalistic and efforts to prevent the Third World from industrialising, said the official. Many agencies such as the World Bank and USAID give preference to projects involving basic needs—a minimum level of food, water and sanitation for all. But some developing countries argue that this can be done in the long term only through industrialisation now, even if basic needs must wait. At a recent meeting of non-aligned nations, it was agreed to oppose the basic needs approach at all world meetings, so nothing will be heard of it at UNCSTD. In the past months, the World Bank has already dropped the phrase from its reports. Similarly, the emphasis will be on developing countries being able to choose technology, including heavy industry, and appropriate technology itself will be left to the NGO forum. The developing countries are particularly concerned about the way the World Bank and the developed countries have been stressing AT to the exclusion of more orthodox technology.

No evidence of innumeracy in UK primary schools: A report of the Schools Council project based on a national survey of 10-year-old pupils and their teachers and head-teachers has found no evidence for innumeracy in schools in England and Wales. The report contradicts widespread media coverage of presumed difficulties with basic maths education (*Nature* 1 February). Preliminary investigation indicated that teachers were concerned about the success of both traditional rote teaching methods and the progressive methods taken up in the 1960s. But the new study found that most schools use a mixture of methods and that teachers were looking forward to a period of consolidation when the majority will "catch up with the vanguard of innovators". Most important, the study finds that children's attitudes towards mathematics have become more positive as a result of new teaching methods, and many parents are surprised that their children actually enjoy mathematics. *Mathematics and the 10-year-old*. Schools Council Working Paper 61. Evans/Methuen Educational £4.25.

National Institutes of Health recommended to take responsibility for low-level radiation research: The US National Institutes of Health should take over responsibility from the Department of Energy as the main agency for research

into the health effects of high and low-level radiation, according to the report of a task force set up last year by President Carter. Although the task force accepts that much of the work carried out by the DoE on the health effects of radiation has been of high quality, it says that public confidence in the results of such research would be greatly improved if it was performed by a health agency. The NIH, it suggests, would be the logical choice.

"The public perceives that a tension exists between DoE's role as the primary sponsor of research into the effects of ionising radiation and its roles as a developer and promoter of nuclear energy and as an employer, through contractors, of workers exposed to radiation during the production of nuclear weapons", the task force report says. It also recommends that the NIH be responsible for chairing a new Interagency Radiation Research Committee, which would be responsible for making sure that the federal government has a comprehensive radiation research programme.

US Army to move nerve gas to new store: The US Army has announced that "in the near future" it will start moving about 9,000 Weteye bombs, each containing more than 300lbs of nerve gas from an arsenal in Denver to a new storage base in Utah. The bombs were originally scheduled to be deactivated but it was later decided to keep them as a deterrent against any Soviet use of nerve gas in the event of war. Plans to move them last year were postponed when three were found to be leaking. The Weteye bombs are the most recent weapon in the US chemical weapons arsenal. Production was halted by President Nixon in 1969, in the middle of anti-Vietnam war protests and after the death of 6,000 sheep in an accident when the gas was tested at a site in Utah.

Dal Lake dying of pollution: Kashmir's Dal Lake—one of the most famous tourist attractions in India—is dying of pollution. A study conducted by the botany department of Kashmir University says that if the present rate of pollution continues, the lake will be dead in the next 80 years. In only the last three decades it has shrunk from 22.64 km² to 16.40 km². The pollutants are the wash-off of fertilisers and pesticides from the surrounding banks, the paddy and orchard fields, and the famous Mughal gardens. There is also sewage released from houseboats, hotels, shops and residential buildings that have mushroomed about this tourist attraction.

The lake is crucial to the transport and economy of the Jammu and Kashmir state. Chief Minister Sheikh Abdullah has recently allocated 200 million rupees for a project to beautify and preserve it, and a team of foreign experts from the Commonwealth Fund for Technical Cooperation has already drawn up a rescue plan. In the first phase of the project, a ring road will be built around the lake to prevent further encroachment. In the second phase, illegal residential colonies that have spread about the lake will be shifted to a new site. Steps will also be taken to plant trees in the surrounding areas, restrict grazing and desilt the lake.

UK Dept of Energy tells it to the schools: Relenting to pressure from school teachers who wanted background teaching material on energy matters, the DE last week published the first in a series of energy notes for schools. The notes, not entirely successfully, attempt to present only non-controversial facts. *Energy a key resource* can be obtained free from Room 1389A, at the Department of Energy, Thames House South, Millbank, London SW1P 4QJ.

Third World Science : continuing our review of science in developing countries, we look at an Indian success | story (below) and the economic and political situation facing a scientist in Ghana (overleaf)

Blue-green algae to fertilise Indian rice paddies

The use of chemical fertilisers can be reduced by a third if algae are applied to rice paddies, writes **Anil Agarwal**

SCIENTISTS of the Indian Agricultural Research Institute in New Delhi are actively promoting the use of blue-green algae as a biological method of fixing nitrogen in rice fields. Dr G. S. Venkataraman of IARI has collected a bank of useful strains of blue-green algae from Indian rice fields. Extensive field trials have been carried out in various parts of India to study the effect of inoculating certain strains of blue-green algae on grain yields of high-yielding rice varieties responsive to inputs of nitrogen.

These experiments reveal that:

- in the total absence of chemical fertilisers, application of blue-green algae can fix about 25-30 kg of nitrogen per hectare per cropping season producing a 10-15% increase in grain yield;
- where chemical fertilisers are used, their dose can be reduced by about one-third and supplemented by blue-green algae to get the same grain yield;
- even when the full recommended levels of chemical fertilisers are used, complementation of blue-green algae is beneficial resulting in higher grain yields.

The increase in yields in experiments conducted by the IARI ranged from 2 to 29% with an average of 7.2%. Besides nitrogen, it seems blue-green algae can also provide crop plants with many useful organic growth substances and vitamins.

The IARI scientists have found that blue-green algae can establish themselves almost permanently in a farmer's field if inoculation is done repeatedly for 3-4 cropping seasons. This produces an appreciable population build-up and no further inoculation is needed in subsequent years, unless some very unfavourable ecological conditions intervene.

The blue-green algae technology is cheap and, therefore, extremely relevant for poor rice farmers for whom the high cost of chemical fertilisers poses a serious constraint in adopting high-yielding varieties. This problem is considered to be an important reason for the slow spread of the green revolution to rice. Most Indian rice farmers are much poorer than the wheat farmers of northern India who have rapidly adopted the green revolution package of practices.

Eighty-seven per cent of the rice area in India consists of holdings of

1-4 hectares and the rest consists of holdings of less than one hectare. Yet rice farmers account for the biggest share of chemical fertilisers used in the country. Rice is the most important and extensively grown cereal in India covering over 38 million hectares.

Emphasising the importance of blue-green algae under these socio-economic conditions, Dr H. K. Jain, IARI's director points out that "if one-third of the nitrogen requirement of the rice crop in many parts of India could be met through the use of blue-green algae, a major constraint in increasing production of this most important cereal will have been overcome".

The IARI estimates that while 25 kg of nitrogen would cost about Rs 100 (approximately £6), the algal material required to provide the same amount of nitrogen to the farmer's field would cost just about Rs 30 (£2), even if it were produced commercially. But if the farmer were to produce his own material the cost could fall to Rs 7 (50p) or even less.

Blue-green algae can be easily produced in any village. The farmer first has to make a simple pit. This could be made of galvanised iron sheets or brick and mortar or by simply digging the earth and lining it with a polythene sheet to hold water. Ten kilograms of soil mixed with 200 gm of ammonium superphosphate is placed in this pit, and the pit filled with 2-6 inches of water. When the soil has settled down, sawdust and the starter culture—a soil based mixture of *Aulosira*, *Tolypothrix*, *Nostoc*, *Anabaena* and *Plectonema*—is sprinkled on the pit. Packets of the starter culture are being sold by the IARI for just Rs 2.50 (15p) each.

The pit must be kept exposed to the Sun and water added daily to it if the rate of evaporation is high. In hot summer months, a thick algal mat can be obtained within a week. The farmer can then allow the water to evaporate and the algae to dry up into thick flakes, which he can store in bags for future use in fields.

This Sun-dried algal material can be stored for long periods and used both directly in the field as well as a starter culture for producing more algae. In the fields, the algae should be applied at the rate of 10 kg hectare one week after transplantation and the field kept waterlogged for at least a

couple of days after algal application. Pest control measures do not interfere with the establishment of the algae, according to IARI scientists.

Several Indian States like Tamil Nadu, Andhra Pradesh, Kerala, Karnataka, Madhya Pradesh and Maharashtra are now taking an active interest in this algal biofertiliser. The department of agriculture of Tamil Nadu, which has taken maximum interest up to now, has included algal complementation in the package of practices it recommends to rice farmers. Farmers are being motivated to use blue-green algae through the mass media and attractive hoardings in villages. Large-scale algal production is being undertaken by paddy experiment stations, state seed farmers, panchayat unions (village councils), and individual farmers.

Interest in blue-green algae has also spread to Burma, Sri Lanka, Bangladesh and Nepal. These countries were recently visited by Dr G. S. Venkataraman on behalf of the Food and Agriculture Organisation to conduct a training course in the production and use of algal biofertilisers. The technology developed in India has been found to be reproducible in some of these countries and algal production has been successfully started by scientists in Burma.

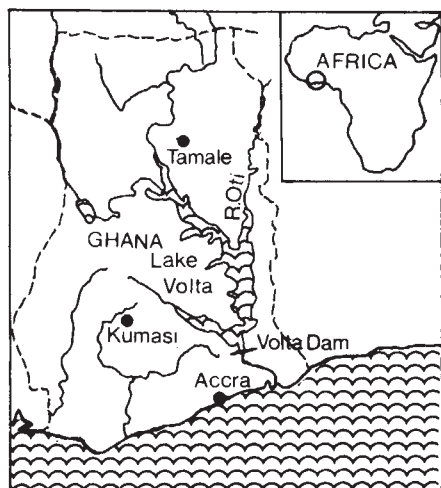
There is still considerable scope for further research in this field. IARI scientists have found, for instance, certain areas in India, like the green revolution region of Punjab, where the inoculated algae do not establish well. Antagonistic indigenous microflora in the soil and other factors seem to play a role but further elucidation is needed.

Dr H. K. Jain also points out that the Chinese and Vietnamese have tried to use the *Azolla* fern to provide biological nitrogen in rice fields instead of blue-green algae. A critical evaluation of the relative merits of the two biological systems under different agro-climatic situations would be useful.

The work on blue-green algae is a part of the All-India Co-ordinated Project on Algae sponsored by the Government of India's Department of Science and Technology in New Delhi. Seven Indian research institutes are looking at various uses of different types of algae like fermentation of marine algae to produce methane in bio-gas plants, production of protein rich algae suitable for human or animal feed and for applications such as sewage reclamation. □

Ghana: science hangs on amid economic chaos

By Joseph Hanlon



GHANA is a small country of 10 million people on the Gulf of Guinea in West Africa. The former Gold Coast, it became independent in 1957 under Kwame Nkrumah. He inherited a colonial economic pattern with its emphasis on the export of primary raw materials. Today, cocoa is still Ghana's largest export, followed by timber, gold, and diamonds.

Nkrumah's government and all succeeding ones have been committed to economic independence. There has been considerable industrialisation and the Volta Dam now supplies much of Ghana's electricity. But Ghana has become even more tied to the world economy.

The military has dominated the government throughout much of the past decade. It overthrew Nkrumah in February 1966. A civilian government was elected in 1969, but overthrown by the military in January 1972. That military government was marked by a spectacular degree of corruption and mismanagement. More than £100 million has been smuggled out of the country by government and commodity marketing board officials.

To meet its commitments, the government simply printed money—since 1972, the money supply has gone up at a steady 80% a year, leading to massive inflation.

Two years ago, students and professionals began a long series of protests, eventually leading to the resignation of General Ignatius Acheampong as head of state in July 1978. A constitutional assembly was set up to lead to civilian government. Political parties were established in January and elections are scheduled for June.

One year after independence Nkrumah's government set up a National Research Council. Ghana also has three universities, with active scientific research at two: the University of Science and Technology at Kumasi and the University of Ghana at Legon, just outside Accra. Local institutions produced 4,234 graduates and diplomates in science and technology between 1951 and 1974. Applied research is under the guidance of the Council for Scientific and Industrial Research in Accra, which has nine research institutes under it.

Research fails to make a real impact

THE Council for Scientific and Industrial Research (CSIR) "has not been able to make the desired impact on the Ghanaian economy" and should be radically restructured, concluded a committee to review CSIR which reported in February 1977. CSIR is Ghana's largest science body. It controls nine research institutes and has an annual budget of 21 million cedis (£3.85 million). It was set up in 1968 following the recommendation of a committee of experts chaired by Sir John Cockcroft.

The 1977 committee was headed by Dr Edward Ayensu, a Ghanaian who is director of the endangered species programme at the Smithsonian Institution in Washington, DC. The other two members were Professor Alastair M. North, vice-principal of the University of Strathclyde, and Dr W. Gerald Matlock of the University of Arizona.

They praised some CSIR units, such as the Building and Road Research Institute, which maintains "balance and relevance". But they found that "the research programmes of many of the CSIR institutes can only be described as an amalgamation of too many unrelated, academically oriented, poorly defined projects. Well written objectives and a realistic time scale are lacking. No attempt has been made to establish priorities and relate projects to country needs. Little co-ordination of the effort has been provided by CSIR and the

fragmented nature of the research increases the costs of both equipment and facilities. New projects continue to be proposed and implemented but the completion rate of the projects is near zero."

Its collective ego badly bruised, CSIR set up a committee headed by its chairman, Professor Albert N. Tackie, to issue a response later that year. Most of Ayensu's objections were simply swept aside. But Ayensu has made his point and fundamental changes have made CSIR more mission oriented.

The Food Research Institute was the target of some of Ayensu's most bitter attacks. Research officers returning from study abroad simply continued their own projects, he said. No one worked together and no one looked at the needs of Ghana. The result was an "undue proliferation of open-ended research projects which are either beyond the capacity of the staff or else are too fragmentary to make an effective impact."

At the Food Research Institute, a new head, J. Maud Kordylas (appointed just before the Ayensu visit), has scrapped almost all previous research projects. Scientists now work in one of four groups: food storage, labour saving rural technology, processing, and consumption. No one works alone; scientists must pick one of the four teams to work with, independent of past training. The storage team actually goes to villages

to collect samples and analyse for moisture, insects, mould, aflatoxins, etc. Traditional fish processing, both smoking and drying, is now being studied. With FAO help, the institute is trying to design an improved smoker with less wastage. The multi-disciplinary team includes an engineer looking at smoking units, a biochemist checking the bioavailability of protein with rat experiments (charring reduces protein availability), and so on.

The Industrial Research Institute also came under attack by Ayensu and it, too, is being substantially restructured. Dr M. N. B. Ayiku, the Institute's head, explained that in the past "we had lofty ideas—finding new raw materials and developing new processes. Now we realise the important thing is for industry to survive. And we realise that for industry to grow, we must have many small industries."

Some changes have not been made, however. CSIR suffers from an only-the-best-is-good-enough syndrome. It still budgets for large amounts of foreign exchange it knows it will not get.

Nevertheless, there is a new enthusiasm at CSIR that, despite administrative and economic problems, the scientists could work together toward the solution of the country's problems. They admit they are just starting. But as Ayiku noted: "If we had concentrated on small industry 10 years ago, by now we would have made a significant contribution." □

Work continues despite strikes and corruption

"No responsible scientist can waste time doing research if they have a family to feed," declared Thelma Dakubu, a University of Ghana physicist. Ghana's economic collapse dominates the life of scientists as much as anyone else. Just surviving takes increasing time, and the research that does get done is plagued by shortages of supplies and equipment. Yet, despite her comments, Dakubu and other scientists continue their work producing results of international standing.

Money dominates Ghana today. Inflation has been running at 100% per year for more than two years. But in a period when prices have quadrupled, government salaries have risen only 10% (most scientists, including university staff, are on government salaries). A typical university lecturer's gross monthly salary is 750 cedis (£130). After tax, car loan payments, and subsidised rent, he or she is left with about 300 cedis (about £1.75 per day) for food, clothing, and other expenses.

Typical prices are: bread, 3 cedis a loaf; a three-pound chicken, 40 cedis; a yam, which will provide basic carbohydrate for a family for two days, 8 cedis; tomatoes, 1 cedi a pound. Prices for other necessities include 7 cedis for a light bulb, 2 cedis for a roll of toilet paper, and 12 cedis for a tube of toothpaste, as the Vice-chancellor of the University of Science and Technology (UST), E. Bamfo Kwakye, admits: "It is not possible for any member of my staff to live on his salary. He must have some other source of income."

Some raise chickens, others have taken to trading or using their cars as taxis. And when they are not earning money, they are out searching for goods. One scientist broke an appointment with me because he had heard of a place in town selling motor oil. My interview with the head of the Atomic Energy Commission came to an abrupt halt because the Army had

arrived to sell cloth. The head, and everyone else at the research centre, queued to buy. Then the interview resumed. The result is clear—less time is left for the lab and classroom. UST is almost deserted in the afternoons. Corruption is also increasing even among scientists. Ghanaians have coined the word 'kalabule' for making a profit through friends.

Some scientists have simply left Ghana. The brain drain has hit the schools, too. More than 4,000 teachers left Ghana between August 1977 and September 1978 and those who remain often attend less than half their classes! The effect can be measured in falling test scores. A survey of "O-level" chemistry exams taken by the top one-third of candidates showed that in 1973, 84% obtained credit or better and 22% of those excellent or very good. Last year, the figures had fallen to 60% and 6% respectively.

Those who stay to do research hit the other half of Ghana's economic crisis—the foreign exchange problem. The military government has diverted foreign exchange to its own uses by granting import licences and letters of credit to government members, friends, and girlfriends. Mercedes Benz cars and luxury goods, as well as expensive military hardware, were imported instead of necessities.

The Council for Scientific and Industrial Research has received no foreign exchange for two years. At UST the bookshop is virtually empty. The most recent copies of journals in the library are months—or even years—old, because subscriptions could not be renewed. Equipment lies unrepaired because spare parts cannot be imported.

At the Atomic Energy Commission's Nuclear Research Establishment—one of the best provided for research units in Ghana—the head of the chemistry department, J. P. H. Brown, said that "sometimes we run out of benzene for months on end". For Dr Sarpong of the UST Faculty of Pharmacy, the worst part is that

"you sit and wait for months hoping to get chemicals and solvents from outside the country. You are never told you are not going to get them."

But the middle class—which includes scientists—has clearly been the hardest hit. "We could not spend all our time talking about professional concerns when the soldiers were mismanaging things," Ayiku said. So they took to the streets. Strikes by students, teachers, scientists, engineers, lawyers, and doctors during 1977 and 1978 eventually led to General Ignatius Acheampong's resignation.

The University Teachers Association, 13 of whose 15 executive officers are scientists, played a leading role in the strike, despite threats that they would be sacked, thrown out of their university owned houses and lose their salaries. Students who should have graduated in July had an entire year of studies squeezed into four months, finishing in December. This put pressure on teachers, too. In combination with the strike, many were not able to do any research last year, and have only recently returned to the lab.

Some scientists are still politically active. Dr Ivan Addae-Mensah, a University of Ghana chemist who succeeds in doing both research and political work, explains the position of scientists this way: "In other countries, people say scientists hide in their shells. As an educated person in a developing country, a lot is expected of you. This makes the scientist in a developing country more aware of his social commitments. It is not because we are scientists, but because we are educated that we have a duty." □

● **Overleaf: Ghana's equipment shortage**



"Money dominates Ghana": consumer goods sell for a fortune and even a yam, the country's staple food, costs 8 cedis, twice the daily minimum wage.

Equipment shortage holds back home-grown science

A GHANA University soil scientist asked me: "Why should we do science the hard way when you have the sophisticated equipment to do it the easy way? You analyse soil and I analyse soil—why should we use different equipment? We are poor, but it is because you made us poor. We were colonies and you are living the easy life and have sophisticated equipment because of what you took from us. You should give us the best equipment with open hands."

Hardware is fundamental to modern science, and it has changed radically in the past two decades. Not only does modern equipment make many routine analyses vastly easier, but many experiments are impossible without it. Indeed, in some fields you cannot publish in prestige journals without using modern, expensive machinery. Equipment came up repeatedly and spontaneously in my discussions with Ghanaian scientists. Not all would argue as strongly as the soil scientist, but most would support the sentiment.

Students returning from abroad find they cannot continue their work because they no longer have access to sophisticated tools. Although this sometimes means they are doing work which is inappropriate to Ghana, many cases can be found where there is a need for modern equipment and researchers must look abroad for help.

Many find this demeaning and feel it puts them at an unbridgeable disadvantage. For example, the Industrial Research Institute is studying refractory clays, which could have considerable commercial importance. The institute's director, Dr M. N. B. Ayiku, said "we are suspicious that if we send the clays abroad for tests, the results will be changed to prevent competition, or will be passed on for foreign companies to exploit".

"It is one thing to acquire sophisticated equipment, and another to maintain it. Maintenance is the limiting factor", argues E. Bamfo Kwakye, Vice Chancellor of the University of Science and Technology (UST). According to a UN Development Programme study, "a large number of instruments are lying idle . . . due to lack of spare parts and maintenance facilities."

Distance is a problem. In Britain, it is possible to telephone a manufacturer's representative who can hop on a train and do a repair quickly, while small pieces of equipment can be sent back to the maker. In the middle of Ghana, neither is possible. So the multi-channel analyser that would be quickly checked on a manufacturer's computer in Britain is laboriously checked one channel at a time in

Accra. In addition, equipment is not tropicalised, which means that parts rot or corrode much more rapidly than in the US or Europe, and it often requires a constant electricity or water supply that cannot be guaranteed. When I visited the University of Ghana, the water had been off for two days. A voltage surge a few months before had been large enough to damage even rugged machinery like air conditioners.

A shortage of technicians is probably the biggest problem. It is the result of a decision during the Nkrumah era to expand rapidly at the top. According to the 'trickle down' theory of development held at the time, a country making a major leap into development with modern industry would pull along the rest of the people, and the benefits and skills would 'trickle down' to them. This failed miserably everywhere. In Ghana, it led to the expansion of the universities and the setting up of centres of excellence, such as the Atomic Energy Commission. Everyone who received a reasonable secondary education was encouraged to go to university, so few people became technicians.

Some university technicians do exist, but as one chemist told me: "We had a first class Ghanaian technician who maintained our gas chromatograph. But his salary was only 6,400 cedis (£1,175) a year, so last year he left for Nigeria. We've appointed two replacements, but neither took the job because they could make more with local electronics firms. So now we must rely on annual manufacturer's tours." Equipment often depends on just one person who knows how to run it. If he is on sabbatical, or leaves his job, the device lies unused.

Other problems have occurred because foreign aid is often 'tied'. For example, British aid usually must be used to buy British equipment, even if it might be more sensible to buy a German or US item similar to equipment already existing in Ghana, with which people are familiar and with spares available locally. This leads to a wide variety of equipment, compounding the original problem. And aid rarely includes maintenance money, service contracts, or even training for local technicians.

Several scientists mentioned the mass spectrometer for potassium-40 dating, contributed by the UK to the Atomic Energy Commission several years ago, but so far hardly used. It is not designed for tropical climates, and fluctuations in the water pressure and electricity have caused frequent breakdowns. Now the manager is in England on sabbatical, and it is locked up.

Problems remain even when equipment and technicians are available. G. V. Addo is a glass blower at the Council for Scientific and Industrial Research (CSIR). With four years' training in Germany, he now repairs complex glassware for universities, hospitals, and research units. CSIR also acquired a test-tube making machine, so that Addo could import glass tubing and make test tubes cheaply. He then found that no one in Britain or Europe would sell tubing to him because they were unwilling to break the monopoly on test tube sales. Only by returning to Europe to negotiate personally, did he obtain offers, from companies in Germany and Czechoslovakia. "The developed countries should be a bit more liberal—we all live in the same world."

But even liberalism involves a measure of control. The government and Unesco worked out an imaginative proposal for a CSIR Scientific Instrument Centre, now being set up with \$1 million of UNDP money. It will have workshops, test equipment, and technicians in fine mechanics, electronics, and calibration. "Look at the centre as a garage for instruments," explained project manager Dr R. G. J. Butler. The project already has eight senior technicians and the electronics workshop is repairing everything from pocket calculators to electrocardiographs. The first step is to bring back into use equipment already in Ghana; it will then serve as a centre for new equipment, which can be maintained on the premises and used by a variety of different groups. The mass spectrometer for the Centre for Scientific Research into Plant Medicine, for example, will be installed at the Instrument Centre rather than the Plant Medical Centre.

The Centre, however, is developing slowly, partly because of UNDP delays. UN agencies fund projects proposed and backed by individual countries, but in this case, the original idea came from Unesco, and was only taken up by Ghana after gentle pushing. Now, however, the "government is 100% behind the project," according to Andrew Taylor, the UNDP programme officer. But Taylor and the UNDP are not. Building a new institution when it is having trouble supporting existing ones is a luxury Ghana cannot afford, Taylor says. Thus, whenever he gets a request from the Centre, it goes to the bottom of the pile and stays there for a while. "We are trying to soft-pedal implementation of the project, hoping that eventually sanity will prevail." □

Dr Hanlon is a science journalist specialising in development matters

The Pope embraces science—if it eschews power



The Pope gave audience recently to the President of the European Physical Society. Here are some extracts of their exchange

HIS HOLINESS THE POPE:

... This meeting this morning is particularly pleasant for me.

In fact, although my personal education has been and still remains in the humanities (I must say that I know very little of your subject), and my concern has always been with philosophical, theological and moral questions, your concerns are by no means unfamiliar to me. It is perhaps somewhat bizarre, but I have always been well-received by physicists, by the people and professors who represent your profession, your specialisation, and while knowing so little about your problems, your science, I have always got on well with them.

In Krakow I always sought contacts with the scientific world and in particular with specialists in the physical sciences, and I found these contacts very fruitful. This will tell you how precious this moment is to me, which evokes so many other meetings, in particular, perhaps that with the "Club of Rome". The results of the works of this Club were well known to us, in Poland, even if circumstances did not permit that meeting to have the aspect of personal exchanges which I would have appreciated so much.

You and your colleagues have been working on scientific problems of great current significance, ranging from very high energies for the study of sub-nuclear phenomena to nuclear fusion, from astrophysical radio-interferometers to synchrotron light. Forgive me if I simply pronounce these words and if I cannot give a personal signifi-

cance to all these expressions and this terminology. But this, I think, is our situation when one lives in so specialised a world: one loses the facility of speaking all possible languages, not only in the linguistic sense of languages but also in the scientific sense.

Thanks to a knowledge of the classical languages (Greek and Latin), one understands a little of what these words mean, but their real significance, the relationship to the reality defined by these terms, that, of course, you must give them. Your society, which includes several thousand physicists from 28 European nations, is at the same time an appeal for the cultural unity of the whole community of European countries.

I do not intend to give you today a thoroughly researched lecture, just a few remarks on the problem, always new and of current significance of the relationship between science and faith. You are, first of all, seekers; I must say that this is a word which is especially dear to me. Seekers! It is proper to raise once again this feature of your work and to encourage the true liberty of our research in its proper sphere and method as "the rightful independence of culture and especially of science" referred to by the Second Vatican Council (*Gaudium et Spes*, n. 59).

I should say that this paragraph of *Gaudium et Spes* is truly important to me. Science in itself is good, since it is a knowledge of the world which is good, created and regarded by the Creator with satisfaction, as *Genesis* says: "And God saw everything that he had made, and behold, it was very good" (*Gen.* 1, 31).

I am very attached to the first chapter of *Genesis*. Original sin has by no means totally altered for the worse the primal goodness. Human knowledge of the world is a means of participating in the science of the Creator. It thus constitutes a first degree of resemblance of man with God, an act of respect towards God, since everything which we discover pays homage to the primal truth.

The scientist discovers hitherto-unknown energies of the universe and puts them to the service of man. By his work he thus makes both man and nature grow at the same time. He should therefore humanise man the more, while respecting and perfecting nature. The universe has a harmony in all its parts and every ecological disequilibrium entails harm to man. The scientist will not therefore treat nature as a slave but, drawing his inspiration, perhaps, from the *Canticle of Creation* of St. Francis of Assisi, he will consider it rather as a sister called to cooperate with him in opening up new ways to human progress.

This path cannot however be followed without recourse to applied science, to the technology which makes scientific research effective. Allow me to refer to my recent Encyclical *Redemptor Hominis* in which I recall the necessity of having a moral rule and system of ethics which will permit man to profit from the practical applications of scientific research, and in which I spoke of the fundamental question of the profound unease of contemporary man. "Does this progress which has man for its author and promoter make human life on Earth



"Science and technology need more of the soul, a new breath of spirit"

Above: Pope John-Paul II greets Professor Antonino Zichichi, President of the European Physical Society

'more human' in every aspect of that life? Does it make it 'more worthy of man'?" (cf. n.15).

There is no doubt that in many respects the technical progress which has been born out of scientific discoveries is helping man to solve such serious problems as food, energy, and the struggle against certain diseases which are more than ever wide-spread in the countries of the third world . . . But it is also true that man, today, is the victim of a great fear, as if he were menaced by that which he creates, by the results of his work and the use which he makes of them. To ensure that science and technology do not become the slaves of tyrannical "will to power", political as well as economic, and to give science and technology a positive direction towards the benefit of man, it needs, as one is accustomed to say, more of the soul, a new breath of the spirit, a fidelity to the moral standards which govern the life of man.

For scientists of different disciplines, and particularly for you, physicists, who have discovered energies of an immense range, it means that you should use all your prestige so that the consequences of science are subordinated to moral standards as regards the protection and development of human life.

A scientific community like yours, which includes scientists from every country of Europe and every religious conviction can cooperate in a unique manner in the cause of peace; science, in effect, supersedes political frontiers and demands world-wide collaboration. Science offers specialists an ideal place for meetings and friendly exchanges which contribute to the cause of peace.

In a concept of science which is ever more lofty, in which understanding is put at the service of humanity in an ethical perspective, allow me to present for your consideration a new step in the spiritual ascent.

There is a connection between faith and science, as you too can testify. The Magisterium of the Church has always affirmed it, and one of the founders of modern science, Galileo, wrote that "Holy Writ and Nature proceed, one and the other, from the divine Word: the one, as being dictated by the Holy Spirit, and the other as the most faithful executrix of God's commands"—so he wrote in his letter of 1613 to Castelli (*Edizione nazionale delle Opere di Galileo*, Vol. V, p. 282).

If scientific research proceeds according to methods of absolute rigour and remains faithful to its proper object, and if scripture is read according to the wise guidelines of the Church, given in the Conciliar Constitution *Dei Verbum*, which are, we

may say, the latest guidelines (previously, there were other similar ones), there cannot be any opposition between faith and science. In the cases where history emphasises such an opposition, this always is due to erroneous positions which the Council has openly rejected, deploring "certain habits of mind sometimes found too among Christians, which do not sufficiently attend to the rightful independence of science. The arguments and conflicts which they spark lead many minds to conclude that faith and science are mutually opposed." (Pastoral Constitution *Gaudium et Spes*, n. 36, 2).

So long as scientists proceed with humility in their research into the secrets of nature, the hand of God will lead them towards the peaks of the spirit . . .

I should like us to be able to continue this contact in the future, finding opportunities and means of indirect contacts—my job and yours do not leave us any other possibility—which would allow me to get to know better the matters with which you are concerned, and those which you would like the Pope to know about. I think that so far we have made some, as it were, preliminary observations. I wish, ladies and gentlemen, that the blessing of the Almighty may descend on your work and yourselves and may give you the strength to contribute to the true progress of humanity, to the health of mind and body, and to solidarity and peace between peoples.

ANTONINO ZICHICHI:

“Holy Father, I should like to thank you on behalf of my colleagues for the great honour you have conferred on us with this audience . . . Physics has the fascination of those things which Galileo called “creatures of God”, and I should like to take this great occasion to put on record that these times in which we live are witnessing an incredible cultural mystification—perhaps the greatest of all times; because the science discovered and revealed by a man of the Catholic faith is today presented as an activity inimical to faith and to the Catholic religion. But science itself says nothing of this.

There are those who neither know nor care what science is, but speak in its name, betraying its aims and confusing fundamental truth. Today we are living in an epoch in which cultural mystification has reached the stage of presenting as scientific ideology something which has absolutely nothing to do with science. Because science means attentive and rigorous studies with predictions and reproducible results. If someone predicts that a stone, instead of falling to the ground, will fall up-

wards instead, then he is not a credible scientist, and there is an end to scientific discussion. But if, on the contrary, an ideology is constructed which in so many words results in the prediction of things that are as absurd as a stone falling upwards, this ideology will still have a great probability of finding numerous adherents, ready, of course, to believe that it has a scientific foundation.

It is the duty of scientists to relate everything to its true position, with clarity. Today we are in fact victims of a great cultural confusion. The role which science ought to play in a future society, in particular in Europe, is that of contributing to the clarification of its own true nature. Science should not be considered the enemy of anyone, and in particular, not the enemy of the values of the Catholic religion.

Science and the Catholic religion cannot be enemies in matters in which their values are identical. Let me cite one example: racism. The Catholic religion is not racist: for a Catholic, black, yellow, or white are all brothers in God. Likewise for a scientist racism cannot exist. If I were to say that I did not believe in a physical law because it was discovered by a negro, I would put myself outside of science. In science, the intelligence of a single individual is the supreme manifesto from which descends his authority. The Catholic religion exalts the value of the individual in a supreme manner: as a child of God. The sanctity of an apostle is not imposed by force of arms, but comes from the sanctity of his actions, just as the worth of a scientist is not imposed by guns, but comes from the intrinsic worth of his intellect.

Science teaches us tolerance, the Church preaches it. The Church condemns deceit, science combats it. If a scientist says that he has discovered a new phenomenon, he is bound to say exactly what he did, in such a way that anyone can repeat his experiment, to find the same result. If the scientist has lied, repeating the experiment will give a different result. And that will be the end of the deceit. The Church preaches charity and love.

And from this you will gain. The scientist who discovers and who teaches knows the meaning of the joy of imparting his knowledge to others. This is a gift which enriches you. The Church preaches universal values. Science discovers universal laws. In this Galileo saw the work of God. The force of attraction of the Earth is expressed by the self-same law that governs the movement of all the stars in our galaxy and of all the galaxies in the cosmos. The light which illuminates this magnificent Throne Room, that which shines in the Sun and is pro-

duced from the stars, all these lights are obedient to the same law.

Science and religion have the attraction of a pure cause. The Catholic religion has the attraction of the highest values of humanity, pity, love for one's neighbour. The exponents of the dominant culture of our time consider stones to be deprived of any cultural dignity. Therefore no one pays attention to them. But in the study of their motion lay the key to reaching the first great example of the universality of physics: universality of motion.

The Greeks did not lack any of the tools which Galileo used to study the natural world: stones, strings and wooden tables. But they lacked the

love for things. Galileo however saw in stones the works of God and turned to them with the humility of a devoted son. From this act of humility was born modern science. From Galileo's stone, today we have subnuclear physics. This tells us that in so small a thing as a proton (if a proton were the size of my finger, I should be as big as the whole solar system) are written all the laws of nature.

Galilean science tells us that we are made out of incredible, not banal, things; and this same concept of matter is understood in a drastically different way from what materialists believed at one time.

And with these sentiments, Holy

Father, I wish to transmit to you the homage of the physicists of all Europe who have come here with me today to bear witness before your Holiness of their devotion and endeavour towards a cultural unification of this old continent in a future in which, throughout all Europe, from the North to the South, from the East to the West, all will be brothers.

TO THIS THE POPE REPLIED:

“I am especially happy that you said that the scientists may be more fortunate than the politicians, who still do not know how to reconstruct the unity of Europe, which you, the scientists, are convinced you can achieve. Well, I am with science, I am with you.”

IN one way, Japan is “like the West only more so”, and in another way, in her retention of an ancient, exotic and formal culture, she differs from us extremely. My wife and I visited Japan last August under the best of possible auspices. We were invited by three friends, virtuosi of molecular biology, two Hiroshis and Mituru, who had worked with me in Berkeley as young scientists and had returned to become full professors at Kyoto, Osaka, and Kanazawa. They took turns in escorting us.

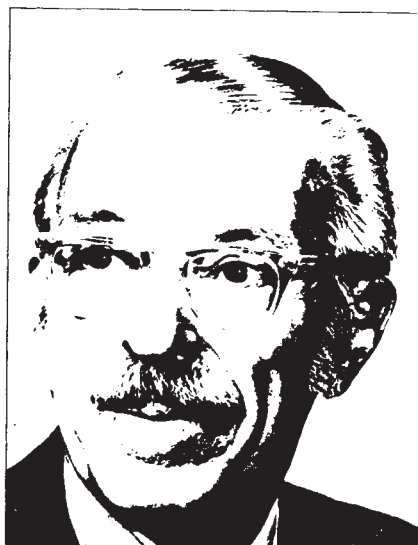
The feats of industrial productivity and the orderly pattern of life in the teeming cities derive from a highly self-disciplined social order that has often been described. We saw many phalanxes of uniformed high school students dutifully marching through the ancient temples and gardens that are the showplaces of Japan. Yet we know that these same young people can erupt in passionate and often violent protest against social injustice. To a nutritionist, of course, it was a source of admiration to contrast their obvious good health and vivacity with the appearance of many of their elders who often were quite small in stature and sometimes seemed subdued.

A nutritional revolution has taken place. Since 1930, rice consumption per capita has declined by 30% while consumption of milk has gone up eight-fold, meat five-fold, and fats and oils, three fold. Lysine, methionine, thiamine, and riboflavin, I thought, must have been major dietary components that had increased. We saw Dr Takaki's house, now moved to an outdoor museum near Kanazawa. His famous experiment in 1887 with sailors in the Japanese navy showed that beri-beri was prevented by adding meat, vegetables and milk to the “official” shipboard diet. It was more than sixty years before cultural and economic

changes made it possible for the lesson to be put to wide use throughout Japan, with the help of electric refrigerators.

We went by car on a fascinating trip to the Japanese Alps. There were thousands of tourists, but we were the only non-Asians that we

East looking west



THOMAS JUKES

saw. We sat on mats in a small hotel for a dinner of rice, fish and spiced vegetables, after which I was beaten easily at “Scrabble”—(in English)—by a 15-year-old Japanese friend. Our room had the traditional sleeping pads on the floor, and a new colour television set on which we watched a baseball game between the Swallows and the Giants.

Our ascent of Mount Hotaka, a

minor summit, next day was along a knife-edge trail thronged with hikers. Conservation of the wilderness is taken very seriously and there was a pleasing absence of litter. As in other countries, the garb of hikers varied all the way from street clothes and sandals to the latest down jackets and cleated boots. The vegetation, especially at lower altitudes, was luxuriant and fascinating, a mixture of exotic leaf and flower forms with familiar ones. Old-world Peacock butterflies, the most glorious of the *Nymphalidae*, floated by to awaken my nostalgia of a childhood in England. Forty Japanese and two visitors sat on the small summit where we ate cold rice for lunch. Far away, a wisp of volcanic steam rose from a green-clad peak.

Many Japanese seem at home in two cultures, their own and ours. It was a matter of course to drive along a high-speed highway, listening to a Mendelssohn symphony on the FM car radio, and looking at men in conical straw hats working in the rice fields. The large roadside restaurants are apt to have three sections: for Japanese food, fast food, and a western type grill. Mituru had received a grant for a P-4 laboratory at Kyoto, but construction of the building could not proceed because of the large number of ancient artifacts that were buried in the soil beneath the proposed site. Preservation of the temples, gardens and palaces of the past is carefully emphasised, and is a good antidote to the onward rush of modern technology.

Everywhere in Japan we were greeted with lavish hospitality and warm friendship. Many Japanese scientists are astonishingly versatile, and characteristically modest and self-effacing. Perhaps, overcoming a language barrier that seems well-nigh insuperable to us, is a hone to the intellect.

correspondence

Is physics levelling off?

SIR,—We thought your readers might be interested in the preliminary results of a quantitative study of the periodical literature of physics and related sciences. We have found that the growth of the literature is substantially slackening, and that the real price of a journal has doubled in the last twenty years.

For our data base, we chose a representative core of journals on the basis of our experience as physicists, and of a survey of the holdings of major libraries as well as analyses of physics literature by other workers in this field. Of the 150 journals chosen for our sample of 1977, 48 existed in 1957. The average rate of birth was approximately five new titles per annum, a new title being a completely new journal, branched title, or translated title.

Most striking of our findings is the recent levelling off in the rate of growth of our sample (see graphs *a* and *b* in the figure) which started in 1971–72 and, according to our estimates for 1978, is still continuing.

How does this result compare with the general vigour of activity in physics? We have examined trends in research support, education and alternative communication systems. With the exception of Japan and West Germany, the general conclusions for major industrialised nations are that a decline began as early as 1966–67 in all three areas. In most cases the support of basic research has fallen more than of applied research.

The available statistics for the UK show that the number of postgraduate studentships awarded in physics and chemistry has remained at approximately the same level since 1966 (1,100 and 1,500 respectively). At the undergraduate level, the number of students taking physics courses declined from the peak 6,980 in 1970 to 5,792 in 1975 (or from one in nine students to one in thirteen). The statistics for the US show similar trends.

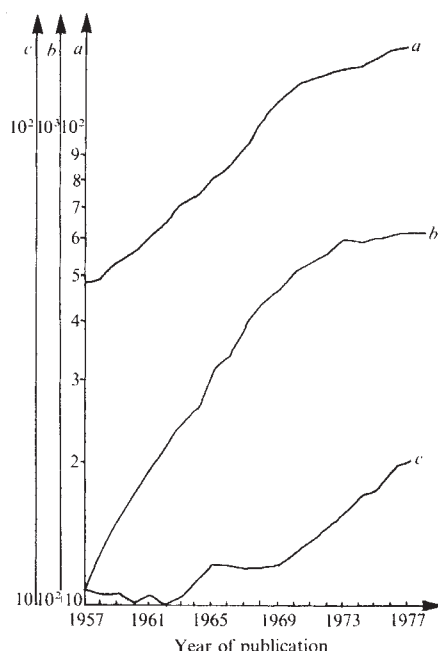
The levelling-off in the rate of growth was helped, but perhaps not caused, by the economic slump of the past five years. Does it reflect the maturity of physics and the first sign of an 'S-curve'? We hope to find out more in further studies.

We have also looked at the price of an average journal. Its cover price was \$24 in 1957 and \$180 in 1977, or in constant dollars (1957=100) \$24 and \$83 respectively. We have used a standard volume for our studies to correct for differences in format, page size, text area and text density. It is defined as a volume of 500 standard pages (210 × 148 mm) with text area of 30 picas in width and 45 lines in depth. The cost of our standard volume to the consumer was \$10.70 in 1957 and \$20.40 in 1977 (graph *c*) in constant dollars (1957=100). However, we have not yet considered in detail changes in circulation, which could have been partly responsible for this doubling in prices. We hope to do this in another report.

It is also interesting to note that the number of new books published in physics (as listed in the British National Bibliography) declined from approximately 360 to 260 in the period 1969–77. In the same period, the average book in physics rose in price from approximately £5 to £15 at current prices but in constant pounds (1957=100) it increased only marginally from £3.40 to £3.90.

SEWERYN CHOMET
ELIZABETH NEJMAN

King's College,
University of London, UK



Levelling-off in physics: *a*, number of journals per year in sample; *b*, number of standard volumes per year in sample; *c*, price per standard volume in constant US dollars (1957=100).

What happened at Hinckley Point?

SIR,—Mr Dunster (29 March, page 393) maintains that I have seriously misunderstood the Hinckley Point incident. I used the short-hand terms 'primary' and 'secondary' for the two cooling systems, so perhaps if I outline my understanding of the incident in a little technical detail, someone can explain where the misunderstanding arises.

● A sea-water pipe feeding the 'auxiliary' cooling system fractured and flooded half of the pump-house. 'Auxiliary' cooling includes the cooling for the concrete pressure vessel and its loss entails shut-down. The probability of this failure is presumably less than 1×10^{-3} per reactor lifetime.

● Due to the failure of a sea-water valve the alternate circuit also failed—thus there was a double fault. The design objective for this double failure presumably gives a combined probability less than 1×10^{-6} per reactor lifetime, ie once in a million years or more.

● Normally the failure of the pressure vessel cooling would not lead to problems of temperature rise due to decay heat in the core, providing the gas circulators continued to operate; but was it not the case that the 'auxiliary' circuit also cooled some component of the gas circuit (the motors perhaps) and that this was a 'common-mode' design error? Mr Dunster states that the 'gas coolant' was 'unaffected'; so I may well have been misinformed.

Whatever the case, everyone insists that the gas temperatures did not rise to unacceptable levels and there was no danger of the fuel cans melting. However, it took three hours to 'jury rig' a fire hose to the 'auxiliaries'—what would have happened had it taken six hours, or twelve? No doubt when the report is published, it will contain details of temperature rise, tolerances, etc, that we may judge for ourselves.

The answer to Mr Dunster's dilemma about information being 'misquoted' is to publish more, not less. Certainly no one will then be led to think officialdom is papering over the cracks. As for 'understanding' the Hinckley Point incident, surely the real significance lies in the non-achievement of design objectives in the crucial area of safety? So far as I am aware, HSE has not made any statement of concern—so perhaps we differ as to what we see as significant.

PETER TAYLOR

Oxford, UK

Sociobiology and environmental determinism

SIR,—Parker (31 August, page 850) has misrepresented my review of sociobiology (*Anim. Behav.* 24, 707; 1976) and maligned me as an environmental determinist. Wilson misunderstands heritability and had misrepresented as success Thoday's failure to split *Drosophila melanogaster* into two species in laboratory experiments. I revealed what Wilson had concealed, both from his own reference where Dobzhansky (*Nature* 230, 289; 1971) stated that "Species formation through genetic divergence . . . has not been observed . . . in experiments" and from *Am. Nat.* (105, 83; 1971) that "Scharloo had already made unmistakably clear that 'several authors in eighteen selection experiments [sic!] have tried in vain to reproduce the(ir) results,'" and so on. With such unreliable treatment of insect evolution (Wilson's speciality) why heed his pronouncements about human behaviour and evolution? That was the substance of my review for which Parker pigeon-holed me with "protagonists of environmental determinism"—a smear contradicted by both Wilson's own reference to my earlier work (*Science* 142, 1436; 1963) and more recent publications (*Ed. Theory* 25, 3; 1975; *Proc. natn. Acad. Sci. U.S.A.* 74, 5193; 1977).

JERRY HIRSCH

University of Illinois,
Urbana-Champaign, Ill.

news and views

Reconstructing the visual image in space and time

from H. B. Barlow

THE first task of the nervous pathways of the visual system is to transmit a suitably coded copy of the retinal image to the primary visual cortex, where it is analysed and the results retransmitted elsewhere. Attention has recently been focused on the nature of this analysis by Hubel & Wiesel (*Proc. Roy. Soc. B.* **198**, 1; 1977) and on the destinations of the retransmitted information by Zeki (*Nature* **274**, 423; 1978) because these interesting matters have become accessible to physiological and anatomical investigation. However recent work in the psychophysics of visual perception suggests that there are fascinating aspects of the initial process of copying the visual image on the cortex that have been overlooked. Furthermore this work may give the key to solving two long standing puzzles of vision. The first is the extraordinary accuracy with which the positions of lines or other marks in the visual field can be estimated, as shown by the eye's ability to align a vernier with an accuracy of about 6 seconds of arc, or to gain an impression of depth from even smaller differences between the images of two eyes. The second puzzle is the effectiveness of cinematography in recreating a moving visual world from a succession of frozen images.

The coded message transmitted from the retina suffers two serious defects, and the new work hints that these are being ameliorated by neural mechanisms in the cortex or even earlier. First, what was a continuous distribution of light intensity on the retina is represented by a set of impulse frequencies in a limited number of nerve fibres in the optic radiation. What can, and what cannot, be reconstructed from such sampled data is well understood, and this is the first point to discuss. The second defect, to be considered

later, is that the image is almost always moving over the retina, and consequently the copy being transmitted to the cortex also moves; why then is it seen as sharp, unsmeared, and still?

Interpolating in space

There is a well known theory that says a continuous function can be completely represented by a finite set of values sampled at intervals $1/2F_{\max}$, where $F_{\max}$ is the highest frequency contained in the continuous function (see for instance, Bracewell, *The Fourier transform and its applications*. McGraw Hill, New York, 1965) and this can be applied to a two-dimensional spatial function such as the retinal image. The spatial frequencies this image contains are limited by diffraction at the pupil to about 60 cycles per degree when the pupil is at its smallest (about 2 mm) in bright light, and to lower values by additional aberrations when the pupil is larger. High spatial frequencies in the image transmitted centrally are also lost as a result of the large receptive fields of retinal ganglion cells (Enroth-Cugell & Robson *J. Physiol. (Lond.)* **187**, 517; 1966), and this is especially important away from the fovea in the near and far periphery of the visual field. Nothing can be done in the cortex to replace the spatial frequencies that have been lost in this way; this information has gone for ever, and in that sense the point sampled representation of the optic nerve cannot be improved. But for some purpose it must be an inconvenient representation. Many people have encountered problems such as specifying the position of the peak of a curve which has only been measured at widely spaced intervals. Surprisingly, this can be done with complete accuracy by the process of interpolation, provided the measurements are not separated by intervals greater than $1/2F_{\max}$, $F_{\max}$ being the highest spatial frequency as before. This process consists of replacing each of the data

points by a curve of the interpolation function, $\sin(x)/x$, of appropriate height, then adding up these overlapping curves at every point. A possible way of achieving this neurophysiologically will be described below. This is relevant for visual physiology because the eye can perform tasks which seem to require a much finer-grained representation than that provided by the optic nerve samples, and the best known examples of such performance are those mentioned above, vernier acuity of 6 seconds and even finer stereoacuity. These are an order of magnitude less than 1 minute of arc or 60 cycles per degree, which is the pupillary cut-off frequency and the highest attainable grating resolution. Of course it has been realised that the attainment of such positional accuracy does not break any physical laws, but it certainly raises the physiological question of how a position can be estimated with greater accuracy than the distance separating two cones in the region of most acute vision. It is, furthermore, a serious problem if one tries to explain perceptual phenomena in terms of representation by single neurones, (Barlow *Perception* **1**, 371; 1972) for how can one explain seeing something placed, say, $1/5$ of the way between the positions of two units?

Now the geniculo-cortical fibres terminate in a layer of granule cells which are extremely densely packed, so densely that there are 30 to 100 times as many granule cells per unit area as there are terminating optic radiation fibres. (Garey, personal communication; Rockel, Hiorns & Powell, *Proc. Anat. Soc. Gt. Brit. Ire.* **118**, 371; 1974.) Along any straight line there are 5 to 10 times as many granule cells as there are sample points from the retinal image; if connectivity from each input fibre to a particular granule cell varied as $\sin(x)/x$, x being the distance to the granule cell, these cells would correctly interpolate the brightness of the image between the samples, and thus a very

H. B. Barlow holds a Royal Society Research Professorship in the Physiological Laboratory, Cambridge.

much finer grained version would be reconstituted.

This 5 to 10-fold improvement would be sufficient to provide the basis for vernier hyperacuity, though stereoacuity is probably too good to be explained in this way. Interpolation could possibly be carried to an even finer level by the more numerous orientationally selective binocular cells in other layers of the cortex, but image movements may also be important, and these are considered next.

Interpolating in time

The fact that the image moves over the retina, either from unintended eye movements, or from failure of the eye to track a moving object accurately, presents a problem. Marshall and Talbot (*Biol. Symp.* 7, 117; 1942) suggested many years ago that movements of the eye, in particular the rapid tremor known as microneystagmus, actually aided resolution, but this idea was always unattractive because it neglected the fact that the eye is a slow instrument that integrates over times of the order of 1/50 s; in such a system rapid movement will inevitably blur the image, and it was eventually shown that resolution was no better with a moving eye than under stabilised image conditions (Tulunay-Keesey *J. Opt. Soc. Am.* 50, 769; 1960). Westheimer and McKee (*J. Opt. Soc. Am.* 65, 847; 1975) therefore asked instead "How well does the eye resist the degradation of performance by image movement?" Their measurements show that resolution stays remarkably good provided the rate of movement is below 2 to 3 deg s⁻¹. Taking an integration time of 1/50 s, this means that the eye can move about 3 minutes within one integration time without marring acuity; that is three times the grating resolution, and at least thirty times the value for vernier acuity! It is clearly the resistance to blurring by movement that requires explanation.

Meanwhile evidence of a quite different kind has been accumulating which suggests that the system has a well developed capacity for temporal interpolation: that is, it is able to represent the position of an object as an almost continuous function of time, even when the image itself is presented at discrete positions at separate instants. This is the basis of cinematography, and until now the prevalent view has been that the gaps in the record are simply ignored or forgiven, or to put it another way, the physiological mechanisms that respond to real motion also respond to discontinuous motion. In contrast the new evidence makes one suspect that there is an actual reconstitution by temporal interpolation of the pattern of activity as

it would have been during the gap between frames. The first evidence for this was contained in some observations made more or less simultaneously by Morgan (*Nature* 526, 639; 1975; *Perception* 5, 187; 1976), Burr (B Sc thesis, Univ. W. Australia, 1975) and Ross and Hogben (*Vision Res.* 15, 1289; 1975) on a well-known phenomenon described by Pulfrich. He observed that displacements in the apparent depth of a moving object result from dimming the view of it by one eye (see Morgan & Thompson, *Perception* 4, 3; 1975, for an interesting account of the history). This dimming is thought to delay transduction by the photoreceptors for a few milliseconds, thereby causing an error in estimating the position of the object in one eye, and a consequent false stereoscopic appearance of displacement in depth. The new observation was that the effect still occurs for an object in apparent motion produced by flashing it stroboscopically at a sequence of positions, as in cinematography. While the authors realised that this was difficult to explain on the classical view, and was for this reason important, they did not bring out the positive implications very clearly, and their interpretations are complicated both by the possibility that the eye tracks the moving object, and by the postulated delay in dimming. These difficulties have been solved to some extent in later work (Morgan & Turnbull *Vision Res.* 18, 1053; 1978; Burr & Ross, *Vision Res.* 19(5), 523; 1979), but a recent demonstration by Burr (*Vision Res.* in the press, 1979) is much simpler and more convincing.

This is an experiment on vernier acuity in which the line segments are displayed stroboscopically at a sequence of stations. Burr used Westheimer and McKee's tricks for controlling the eye movement problem; they curtailed the duration of movement to 125 ms so that reflex following movements would not have time to start, and they randomised the direction of motion to avoid any possibility of anticipatory movements. Like Westheimer and McKee, Burr found that offsets were detected with practically the normal accuracy in spite of the movement of the object; in addition he made the compelling new observation that an illusory displacement occurs if the line segments are accurately aligned in space, but are displayed with a slight delay in one sequence relative to the other. The line segments are not actually displaced; it is the positions estimated by temporal interpolation that are displaced relative to each other, and Burr showed that the accuracy of detecting this equivalent displacement was not far short of the

accuracy for a real displacement. It is hard to avoid the conclusion that something occurs equivalent to the reconstitution of the spatial patterns of activity at moments intermediate between the stroboscopic flashes, for how else could the pair of patterns corresponding to each line segment be seen as occupying different positions?

It has been suggested that temporal and spatial integration would smooth out the discontinuities of the stroboscopic sequence, but they would also mar resolution. It is in fact the demonstration that acuity is preserved for objects in motion, and that time and position produce interchangeable effects, that forces one to the conclusion that a mechanism for temporal interpolation exists. Some process of integration must occur, because one experiences a single moving object, not a succession of separate stationary ones; but if this is integration in the mathematical sense, it must be done along a trajectory in space and time matching that of the moving object, and not separately or sequentially in the two domains. Burr did a nice variant of his experiment which shows that the trajectory of the movement must be established before high resolution can be obtained. If the moving lines were shown in a synchronous sequence for the early positions, but with a delay for the final positions, the misalignment was well detected. If, on the other hand, this early part of the sequence was omitted, leaving only the final part, it was very poorly detected, though a real, spatial, misalignment was detected with high accuracy in these circumstances.

No hypothesis can be advanced as to how this might be achieved neurophysiologically, but it is interesting to hear that Cynader, Gardner and Douglas (*Frontiers Vision Res.* in the press, 1979) have made observations on cells in the cat's visual cortex which imply that time and position can play interchangeable roles in determining the apparent position of a stimulus object.

To summarise, the psychophysical experiments reviewed here suggest that, before any analysis of the retinal image occurs in the visual cortex, it is reconstituted in a finer-grained form than the number of optic nerve fibres would lead one to expect; furthermore the neural mechanisms that do this appear to incorporate special features for gaining high positional accuracy for moving images. It is a neat trick for the system to ameliorate defects in the neural representation of the visual image by combined spatio-temporal interpolation: the evidence for it is convincing and one would like to hear further details on how it is done. □

The productive oceans

from P. M. Holligan

PHOTOSYNTHETIC phytoplankton are the primary producers in the oceans with around 80% of the total photosynthetic carbon fixation occurring in the open ocean, the remainder probably in upwelling areas and shallow seas (Ryther *Science* **166**, 72; 1969). In the nutrient-poor tropical and subtropical waters nutrients rather than light are considered to limit primary production, as levels of dissolved organic phosphate and nitrate in the surface layers of the ocean are generally close to or below the limits of detection. In this issue of *Nature* on page 210, J. C. Goldman, J. J. McCarthy and D. G. Peavey draw together much recent information on phytoplankton growth rates in the oceans and from this synthesis propose the simple concept that although in oligotrophic waters the supply of nutrients, nitrogen in particular, determines the biomass or standing crop of phytoplankton, and therefore productivity, the growth rate of the individual plant cells remains close to the maximum despite an apparent shortage of nitrate and inorganic phosphate.

Phytoplankton growth in oligotrophic waters has only been understood in detail since the institution of formal analysis of the concepts of nutrient uptake and regeneration (Dugdale *Limnol. Oceanogr.* **12**, 655; 1967). Subsequent research on this topic has been mainly concerned with the dynamics of algal growth in continuous culture systems and interpretation of such experiments in relation to field observations on nutrient distributions and the physiological characteristics of natural phytoplankton populations.

Goldman *et al.*'s important conclusion stems from two basic observations: first, that measurements of the carbon, nitrogen and phosphorus content of natural populations of marine phytoplankton consistently give ratios close to 106 : 15 : 1 for the three elements and, second, that in their own experiments with continuous cultures of different species of algae these ratios are only attained at maximal growth rates. The central theme in the interpretation of the data is the analogy of steady state growth both in the continuous cultures and in surface oceanic waters. For the latter it is assumed that regenerative processes are the principal source of nutrients, providing a continuous supply of ammonium, urea and other organic forms of both nitrogen and phosphorus, and also that

the spatial and temporal distributions of phytoplankton biomass are effectively uniform.

The basis for this approach to phytoplankton growth has been established over the past few years by several research groups. For example, Thomas (*Limnol. Oceanogr.* **15**, 380; 1970) and Morris *et al.* (*Limnol. Oceanogr.* **16**, 859; 1971) provided evidence to suggest that phytoplankton in nutrient-poor waters is not nitrogen deficient; the similarity between the oligotrophic oceanic environment and steady-state culture systems was first pointed out by Eppey *et al.* (*Limnol. Oceanogr.* **18**, 534; 1973); and the possibility that grazing stress influences nutrient utilisation by phytoplankton was evaluated by Walsh (*Limnol. Oceanogr.* **21**, 1; 1976). But, as Goldman, McCarthy and Peavey indicate, acceptance of their simple concept of primary production in the oceans will depend on further investigation both of the ways in which nutrients are made available in the euphotic zone and of the physiological responses of plant cells to changes in the nutrient regime.

Although there are uncertainties about the details of nutrient recycling in surface oceanic waters, the general principles, in terms of the role of zooplankton and bacteria and also the relative time scales for the regeneration of nitrogen and phosphorus, are probably fairly well understood. This does not seem to be true, however, for the processes determining the transfer of nutrients into the surface layer. Thus, the influence of small-scale, vertical mixing across the thermocline on primary production has been recognised only recently (McGowan & Hayward *Deep-Sea Res.* **25**, 771; 1978), and considerable horizontal heterogeneity of biological as well as physical properties has now been described by Wiebe *et al.* (*Deep-Sea Res.* **23**, 695; 1976) and Shulenberger (*Deep-Sea Res.* **25**, 1193; 1978). Short-term variations in the concentrations of regenerated nutrients, that have been related to the vertical migration of zooplankton (Beers & Kelley *Deep-Sea Res.* **12**, 21; 1965), may also be produced by the combination of physical and biological processes at the level of the deep chlorophyll maximum which is a characteristic feature of tropical oceanic waters (Yentsch *Deep-Sea Res.* **12**, 653; 1965; Venrick *et al. Fish. Bull.* **71**, 41; 1973; Gieskes *et al. Neth. J. Sea Res.* **12**, 195; 1978).

Any fluctuation in the supply of nutrients raises the question of how phytoplankton cells and populations adapt to such changes. This complex problem is now attracting a great deal of attention, and the types of response shown by stressed cells to increased levels of nutrients range from the rapid stimulation of uptake rates (McCarthy

& Goldman *Science* **203**, 670; 1979) to more gradual 'shift-up' phenomena which lead to higher maximal growth rates (see Dugdale *The Sea* Vol. 6 (ed. Goldberg) 789; 1977).

One aspect of the ecology of oceanic phytoplankton that has been very neglected is their taxonomy. Apart from coccolithophorids (Okada & McIntyre *Micropaleontology* **23**, 1; 1977) the dominant forms are probably small (< 10 µm diameter), naked flagellates. These are difficult to culture or to preserve for direct microscopic and, as a result, little is known about the physiology of growth or of the distribution of the important species in the ocean. Furthermore, investigations of nutrient utilisation by mixed populations (Tett *et al. J. mar. biol. Assoc. U.K.* **58**, 923; 1978) or of nutrient competition between species (Tilman *Ecology* **58**, 338; 1977) have only been carried out for coastal and freshwater phytoplankton communities. The need for information about the biology of individual organisms is emphasised by the recent discovery by Waterbury *et al.* (*Nature* **277**, 293; 1979) that unicellular cyanobacteria (blue-green algae), 1–2 µm in diameter, are widespread in the sea.

It remains to be seen whether the hypothesis proposed by Goldman *et al.* is an accurate description of phytoplankton growth in the tropical oceans within the scales of variability that are known to exist, and whether the same ideas can also be applied to stratified continental shelf and temperate oceanic waters. □

Carbon in the sea

from Egon T. Degens

THE chief goal of a recent conference on Carbon in the Sea* was the evaluation of recent base-line studies in the Atlantic and the Pacific in respect to their contribution to understanding the global carbon cycle.

In the sea, carbon is partitioned into an oxidised and a reduced carbon pool. Bicarbonate ion is the principal species in the oxidised pools, dissolved organic matter in the reduced pool. The sizes of the two reservoirs are 35×10^{18} and 1×10^{18} g C, respectively. These figures

*A Conference on Carbon in the Sea was held in Hamburg on 14–16 March, 1979. It was organised by the staff of the SCOPE International Carbon Unit at Hamburg University, 2000 Hamburg 13, FRG, from whom further details may be obtained. SCOPE is the Scientific Committee on Problems of the Environment of the International Council of Scientific Unions (ICSU). SCOPE's project on Biogeochemical Cycles includes studies on the cycles of carbon, nitrogen, sulphur and phosphorus and is gradually being directed towards the interactions of these cycles. It is carried out with support from UNEP, Unesco and several national and international organisations. Further information can be obtained from the SCOPE secretariat, 51 Boulevard de Montmorency, 75016 Paris, France.

P. M. Holligan is at the laboratory of the Marine Biological Association, Plymouth.

are quite substantial considering that the estimated total biomass on Earth is only 0.6×10^{18} g C. In the light of these data it is conceivable that the ocean is a potential sink for the man-made CO_2 which is released into our atmosphere at the rate of around 10^{16} g C annually at present. Such a comparison, however, can be misleading because the air-sea boundary operates as a kind of bottleneck which limits the molecular exchange between air and water.

The conference demonstrated that there is not just one but a series of bottlenecks restricting the flow of carbon from one compartment to the next. On the other hand however, there are also mechanisms at work that accelerate the carbon flux in the marine environment.

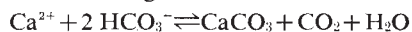
A highlight in the discussion on dissolved organic matter was the astounding progress in the field of analytical techniques. Just one millilitre of seawater is all that is needed now to measure free and combined amino acids, sugars, amino sugars and uronic acids in a matter of an hour and this without desalting the sample. Improved methods were also reported for hydrocarbons and fatty acids. Last year's base line studies in the Atlantic and Pacific using these new analytical tools revealed that dissolved organics concentrate around pycnoclines and that in the euphotic zone there are substantial diurnal fluctuations in dissolved organic carbon, amino acids and sugars. These phenomena may be biologically controlled or could be a result of remineralisation; the precise mechanism still escapes us.

So it seems that the past approach to trace-organic analysis, that is the analysis of seawater and the suggestion of a feasible process to explain the presence of substance X, should be replaced by a consideration of the known biochemistry of cellular systems and attempts should then be made to detect these biochemical intermediates. This is now well within the reach of our analytical techniques.

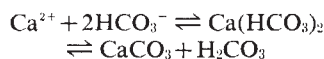
The data on hydrocarbons tell a grim story. In the centre of the Pacific and the Atlantic the hydrocarbon content of seawater is appreciable, of the order of $50 \mu\text{g l}^{-1}$; but these are hydrocarbons of a geological and not a recent biological source. We are still left with the question: are these hydrocarbons relics of seafloor seepage or of former oil spills? More work is needed.

The working group on carbonate equilibria came up with a number of intriguing results which may have direct relevance to the CO_2 build-up in our atmosphere. For instance, calcite particles in the ocean mixed layer may be coated with carbonate films. These films seem to be metastable relative to

ordinary calcite or aragonite and start to dissolve in response to a slight increase in CO_2 content in the ocean mixed layer. Uptake of dissolved CO_2 by reactions with solids in the sea at a water depth far above the aragonite saturation line is strongly supported by the experimental data and may account for some of the 'missing CO_2 '. Another point was made that shell-forming organisms do not generally deposit their CaCO_3 along the familiar route



but by way of a template-induced mechanism using carbonic anhydrase which operates on a neutral H_2CO_3 molecule as substrate



A slight increase in biomineralisation—which so far escapes routine analysis because it is not accompanied by the release of CO_2 and carbon isotope fractionation—may thus be

Egon T. Degens is in the Geologisch-Paläontologisches Institut, University of Hamburg.

When to be incestuous

from Robert M. May

In sociobiology's quest for universal attributes of human behaviour, taboos against incest have received much attention.

The exact degree of consanguinity at which a mating is deemed incestuous varies significantly among existing human societies, but parent-offspring and brother-sister matings are forbidden in most. These are matings between relatives whose 'coefficient of relationship', r , is $r = \frac{1}{2}$; r expresses the probability that a given gene in one individual will be present in a designated relative (that is, it measures the fraction of your genes that are present in your relative, or, equivalently the extent to which the relative is genetically identical to you: Hamilton *J. theor. Biol.*, **7**, 1, 17; 1964; see also *Nature* **260**, 9; 1976). Incest between siblings or parent and offspring does occur in modern societies, although at a low rate that is very hard to determine. One estimate, from the records of charitable institutions in Italy, gives a ratio of one such incestuous birth per 10^3 births, but this is, by its nature, an underestimate (Cavalli-Sforza & Bodmer *The Genetics of Human Populations*, Freeman and Co., San Francisco, 1971). A study in Michigan by Adams and Neel (*Pediatrics*, **40**, 55; 1967) suggests a ratio of one in 10^4 births. In general, the obvious bias against reporting

another sink for the 'missing CO_2 '.

The working group on metal organic complexes showed that considerable progress has recently been made in identifying dissolved trace metal species in the sea by means of differential pulse anodic stripping voltammetry. This method requires no sample pretreatment, can be made directly on board and has the further advantage that ligand numbers and conditional stability constants of certain complexes can be determined. First data on seawater indicate that the operative mechanism of chelate formation with heavy metal ions consists in ligand exchange between an alkaline earth chelate and a labile heavy metal complex species (for example chloro-, or carbonato- complexes).

The general conclusion is that improved analytical techniques and instrumental design have added a new dimension to ocean research. Preliminary studies on all fronts suggest that the oceans are the main sink for the man-made CO_2 in our atmosphere.

makes for unreliable statistics on the number of births from incestuous matings, and it has been argued that their incidence is higher, both in isolated and in urban communities, than public pieties would suggest. It may be indicative that the Rape Squad of the Philadelphia Police Department has recently found cause to create a special unit to deal with incestuous rape.

In many ancient civilisations, incestuous marriage between siblings or parents and offspring was encouraged or even mandated for the ruling class. This was the case, for example, for the pre-hellenic Egyptian Pharaohs, the Incas, and the Ptolemaic and Scllucid dynasties. In ancient Iran, incestuous marriage was urged in the Zoroastrian scriptures, and was regarded as an act of piety (Garsoian, *Handes Amsorya Z. Armen. Philol.*, **90**, 177; 1976; Note 60 and references therein). This religious encouragement derived in part from the Zoroastrian mythology, whereby the human race sprang from three consecutive consanguineous unions, between Ohrmazd and his daughter Spandarmat, between Spandarmat and her son, and finally between the son and daughter thus produced (which, by my calculation, gets the race off to a start with an average co-

Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

efficient of relationship $r=15/16$ among the members of the next generation). Historical records confirm the incestuous marriages of the royal families of ancient Iran, but as usual are mute about the common folk. It seems, however, unlikely that incest of the first degree was generally frowned upon in a society whose religion so explicitly encouraged it (Garsoian *op. cit.*). On the American frontier in the eighteenth century, and in isolated parts of rural Britain in older times, it was not unheard of for the eldest daughter to succeed her mother as chatelaine and wife to her own father. These facts seem to have been overlooked by many sociobiologists, who typically are transmogrified biologists or anthropologists, rather than historians. It remains true, however, that the taboo against incest of the first degree, although not universal, is among the strongest and most widespread taboos in human societies.

After sibs and parent-offspring, the next closest degree of consanguinity is that between uncle and niece, aunt and nephew, double first-cousins or grandparents and grandchildren. Here $r=\frac{1}{4}$. Such marriages are forbidden, or subject to special dispensation, in most contemporary societies. The records of Roman Catholic dispensations in Catholic Europe and Latin America provide an enormous amount of information on the incidence of consanguineous marriages in these societies, and show that uncle-niece and aunt-nephew marriages comprise from 0.1% to 0.01% of all marriages (Cavalli-Sforza & Bodmer, *op. cit.*). Such unions are actually encouraged in some cultures. In Andhra Pradesh, in South India, certain castes favour uncle-niece marriages, which comprise more than 10% of the total.

In first-cousin marriages, the partners' coefficient of relationship is $r=\frac{1}{8}$. As we shall see below, although the chance of deleterious recessive alleles being expressed in such matings is less than for those with $r=\frac{1}{4}$ or $\frac{1}{8}$, the genetic cost of inbreeding of this degree is still quite substantial. Even so, such consanguineous marriages seem to be encouraged in many societies, and possibly have historically been encouraged more often than discouraged. In the USA today, marriages between uncle-niece, aunt-nephew and first-cousins are forbidden by law in roughly half the states; in others, first-cousin marriages require special permission from state authorities. In effect, the Roman Catholic church requires a formal dispensation before permitting marriages between individuals whose coefficient of relationship exceeds $r=1/32$. The customs followed in granting such dispensations are revealing: in Catholic Europe before the nineteenth century, first-cousin mar-

riages were rare and strongly discouraged; following Napoleon's abolition of the right of primogeniture, first-cousin marriages increased throughout the nineteenth century, probably to help keep property concentrated (Bodmer & Cavalli-Sforza *Genetics, Evolution and Man*, Freeman and Co., San Francisco, 1976). In Catholic Europe and Latin America, Roman Catholic first-cousin marriages in the twentieth century comprise about 1 to 2% of the total of all marriages. In Guinea in 1955, a study by Cantrelle and Dupire (*Population* 19, 529; 1964) showed 19% of marriages to be between first-cousins and fractions as high as 30% have been recorded in South India. In Japan, "first-cousin marriage is encouraged and, in certain areas or social strata, up to 10% of the marriages are between first-cousins." (Bodmer & Cavalli-Sforza, *op. cit.* page 365).

In small groups geographically or culturally isolated, there is typically and understandably a significant amount of inbreeding. Thus the average coefficient of relationship between marriage partners for the Hutterites is $r=0.04$, for the Dunkers (a religious isolate in Pennsylvania) is $r=0.05$, for the inhabitants of Tristan da Cunha is $r=0.07$, and for the Samaritans (in Israel and Jordan) is as high as $r=0.09$. Interestingly, other isolates that have existed for a longer time, such as Polar Eskimos, have social rules that keep the average degree of inbreeding around $r < 0.006$, which is not much larger than that for most western societies (where r is typically less than, or around, 0.002).

The above catalogue makes it clear that the genetic costs of inbreeding can often be counterbalanced by economic or other advantages, whose details depend on the way the society is organised. Thus even for incest taboos in human societies, in general we have to weigh advantages against disadvantages in specific situations, and truly universal patterns of behaviour are hard to come by. This is just one particular aspect of a larger problem that E. O. Wilson himself has emphasised, in a broader context (in *Theoretical Ecology: Principles and Applications*, Blackwell, Oxford, 1976). He observes that, were no genetic costs incurred by inbreeding, "there should be a strong selective advantage for inbreeding combined with recognition and cooperation among kin. . . . Such a breeding system would facilitate the evolution of extreme altruism and cooperation among the family members, since a large fraction of genes are expected to be shared through common descent by the altruists and the recipients of the altruism." Wilson then poses, as a major question for sociobiology to answer, "how are the gains

through inclusive fitness and losses through inbreeding depression balanced; and in which life history strategies is this balance struck so as to increase the likelihood of more intense inbreeding and social life?"

An elegant paper treating such a trade-off, within the confines of a simple conceptual model, is by Bengtsson (*J. theor. Biol.* 73, 439; 1978). He considers a sexually reproducing animal that faces a dilemma. It can stay in its local area, risking that it will mate with a close relative and produce offspring suffering from inbreeding depression. Or it can migrate, avoiding inbreeding, but incurring a higher mortality rate. Specifically, let the fractional increase in mortality, associated with migration, be m (the death rate of migrants, relative to stay-at-homes, is $1-m$); this quantity m can include other factors, such as an effective decrease in fertility associated with time lost in migration, or in establishing rank in a new dominance hierarchy. Further, let the cost of inbreeding be i , in the sense that the number of inbred offspring surviving to reproductive age, relative to the number of offspring produced by unrelated parents, is a fraction $1-i$. Assuming the stay-at-homes must thereby mate with their sisters, Bengtsson shows it will pay males to incur the costs of migration unless

$$m > (3/2)i$$

More generally, if stay-at-homes mate with females whose coefficient of relationship is r , males will do well to migrate unless the additional mortality exceeds

$$m > (1+r)i$$

This formula has an intuitive explanation: the relative cost of migration is, by definition, m ; the cost of inbreeding with a partner who shares r of your genes is i , and this cost accrues both to you and to that fraction r of your genes carried by your partner (hence the factor $1+r$ on the right-hand side).

Both here and in the earlier discussion of incest in human societies, the quantity i which measures the loss in genetic fitness due to inbreeding is central. It can be estimated if we know the number of 'lethal gene equivalents', n , carried (in recessive form) by the average individual. This number n is obtained by adding up for all deleterious recessive genes, the average probability of their occurrence times the decrease in fitness they produce in the homozygous state; in this accounting, one recessive lethal is equivalent to 10 recessives that each produces a 10% decrease in the fitness of homozygotes (for example 1 in 10 such individuals dies). For human populations, a classic estimate of the average number of lethal equivalents per person drew on

earlier work to give n in the range 3 to 5 (Morton, Crow & Muller *Proc. natn. Acad. Sci. U.S.A.* **42**, 855; 1956). A more recent figure, based on 18 studies analysed by Schull and Neel (*The Effects of Inbreeding on Japanese Children*, Harper and Row, New York, 1965) and Cavalli-Sforza and Bodmer (*op. cit.*), is $n \approx 2.2$. This number is heavily weighted with data from Japanese populations, and it may be low. Nonetheless, as a very rough estimate we may assume that the average person carries the equivalent of two recessive lethals. Genealogical discussions of inbreeding commonly employ Sewall Wright's 'inbreeding coefficient', F , which is defined as the probability that an individual receives at a given locus two genes that are identical by descent. As F gives the probability for a recessive lethal gene to be expressed homozygously due to inbreeding, it is of direct relevance to a quantitative estimate of i . Ignoring possible complications arising from inbreeding in previous generations, we can relate r and F by noting that if the parents have a coefficient of relationship r , the progeny have $F=r/2$; if a given gene is inherited from the father, there is probability r that the mother carries the gene, and a further probability $\frac{1}{2}$ that it (rather than its diploid complement) is inherited from the mother. Thus $F=\frac{1}{4}$ for the progeny of brother-sister or parent-offspring matings, $F=\frac{1}{8}$ for the offspring of uncle-niece, $F=1/16$ for the offspring of first-cousins, and so on. More generally, if the parents themselves have inbreeding coefficients F_0 , as well as being related with a coefficient r , their offspring will have an inbreeding coefficient $F=r(1+F_0)/2$.

Assuming that all the genetic loci that sum to give the number of lethal gene equivalents may be treated as behaving independently and randomly, the probability for an individual with inbreeding coefficient F to survive to maturity, compared with an individual with $F=0$, is

$$1-i=\exp(-nF)$$

This is simply the zero term in a Poisson distribution, and holds true regardless of how many small bits are added together to get n .

Using $n \approx 2.2$ for the number of lethal equivalents, and bearing in mind the uncertainties about this number that were mentioned above, we estimate that about 42% of the offspring of sister-brother or parent-offspring matings die before reproductive age, compared with the offspring of other similar but unrelated parents. The corresponding cost for uncle-niece and other $r=\frac{1}{4}$ matings is 24%, and is 13% for first-cousin and other $r=\frac{1}{8}$ unions. This is consistent with a study reported

by E. O. Wilson (*On Human Nature* Harvard University Press, 1978) on 161 children born to Czechoslovakian women who had sexual relations with their fathers, brothers, or sons. Of these children, 15 were stillborn or died within the first year of life, and 40% of the survivors suffered from severe physical or mental disorders that would have diminished their Darwinian fitness to around zero in a simple society. A control group of 95 children born to the same women through non-incestuous relations were on the average as normal as the population at large; 5 died during the first year of life, and only 5 others had significant physical abnormalities.

Information about inbreeding depression in natural populations of non-human animals is scant. Bulmer (*Heredity* **30**, 313; 1973) has analysed some of the extensive data on great tits, and suggested that for brother-sister matings among these birds i is around 0.25. Bengtsson quotes data from laboratory experiments on Japanese quail, where the inbreeding depression factor i for sibling unions is around 0.66; this study includes the effects of lowered fertility in the offspring as well as the increased mortality, and may therefore be more typical of the effects of sibling mating in natural populations.

Taking $i=0.4$ as a representative figure for sibling unions, we see from the above formula that males should migrate rather than stay at home to mate with their sisters, unless the additional mortality associated with migration exceeds 60%. Clearly the numbers and assumptions can be juggled to tip the scale either way. Moreover, the model can easily be made more complicated. For example, even in a stable and benign habitat, it is to the advantage of a parent to disperse some fraction of its progeny as colonisers, and to keep a fraction home to preserve possession of the family site; models of this kind (Hamilton & May *Nature* **269**, 578; 1977) can be combined with Bengtsson's, with inbreeding depression providing an added impulse toward dispersal.

In this way, piece after piece can too easily be added to build an enchanting castle that rises free, constrained to earth with too few anchorlines of fact. What we really need, for both human and other animal societies, is a larger body of systematically compiled information about kinship structure, the biological costs of inbreeding (as determined by the number of lethal gene equivalents), the mortality associated with migrating to a new group, and other like quantities. It remains clear, however, that there will always be circumstances when (as Ogden Nash put it), "vice is nice, but incest is best." □

Splicing *in vitro*?

from T. Barrett

IN a recent paper by Plotch *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 1618; 1979) evidence is presented in support of a unique RNA processing step in influenza virus. They have found that the virus transfers a 5'-terminal cap region from a host mRNA to its own mRNA. Influenza, a segmented negative strand RNA virus, is unique among the non-transforming RNA viruses in that it requires a functional host cell nucleus for its transcription *in vivo*. Shortly after infection the virus genome is transcribed by a virus-associated transcriptase into complementary RNA (cRNA) which acts as the virus mRNA. It has long been known that inhibition of host DNA transcription by actinomycin D early in infection prevents replication of the virus (Barry *et al.* *Nature* **194**, 1139; 1962). Later, the drug α -amanitin was shown to inhibit an early increase in RNA synthesis observed in cells infected with influenza virus (Mahy *et al.* *Proc. natn. Acad. Sci. U.S.A.* **69**, 1421; 1972) which suggested that a species of host-coded mRNA was necessary for replication of the virus. Studies using amanitin sensitive and resistant cells showed conclusively that an active host cell DNA-dependent RNA polymerase II is the specific nuclear function required by the influenza virus (Lamb & Choppin *J. Virol.* **23**, 816; 1977; Spooner & Barry *Nature* **268**, 650; 1977). In addition the observation by McGeoch & Kitron (*J. Virol.* **15**, 686; 1975) that dinucleotides stimulate the virus transcriptase *in vitro* by acting as primers for RNA transcription was strongly suggestive that *in vivo* a host cell RNA was required to initiate transcription. This idea was strengthened by the report by Bouloy *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 4886; 1978) that a cellular mRNA, specifically β -globin mRNA, can also act as a primer for the transcription of influenza virus RNA *in vitro*.

The same group (Plotch *et al.* *op. cit.* 1979) have now extended their work on this priming reaction and have produced convincing evidence that the 5'-terminal cap of the globin mRNA is transferred, along with 10-15 globin nucleotides, to the 5' end of the virus mRNA during transcription *in vitro*. In contrast to other negative strand viruses, no endogenous capping activity can be detected in the influenza virus *in vitro* (Plotch *et al.* *J. Virol.* **28**, 75; 1978).

T. Barrett is in the Department of Pathology, University of Cambridge.

The globin mRNA-primed influenza cRNA directed the synthesis of virus-coded proteins in a cell-free translation system and, unlike the dinucleotide (ApG)-primed virus cRNA, was inhibited by pm⁷G which specifically inhibits the translation of capped mRNAs. Enzymatic or chemical removal of the cap from globin mRNA abolished its stimulatory activity for the virus transcriptase but when the mRNA was recapped, approximately 60% of the stimulatory activity was restored. Globin mRNAs labelled only in the cap were prepared by recapping β -eliminated mRNAs with [α -³²P]GTP and unlabelled S-adenosyl methionine. Using this mRNA as a primer and unlabelled nucleoside triphosphate precursors, the virus mRNAs which were synthesised contained a [³²P]-labelled 5'-terminal cap. After removal of their 3' polyadenylated tails with ribonuclease H, the globin mRNA-primed cRNA segments were 10–15 nucleotides longer at their 5' ends than the ApG-primed cRNA segments, indicating that, in addition to the cap, about 10–15 nucleotides were also transferred from the globin mRNA to the virus cRNA. Influenza virus seems to be unique in possessing this cap transferring activity and no other known virus transcriptase uses eukaryotic mRNAs to initiate RNA synthesis in this way.

Plotch *et al.* (*op. cit.* 1979) propose that AG, AGC or AGCA sequences in the β -globin mRNA hybridise to the 3' end of the virus genome RNA (vRNA) template and serve as a primer sequence in the same way as the primer ApG hybridises with the UC residues at the 3' end of each vRNA segment. The β -globin mRNA would then be cleaved at the 3' side to allow transcription of the vRNA. A close interaction of the cap and the primer sequence is expected since the cap is required for priming. They suggest that the cap may be responsible for making the primer sequence in β -globin mRNA 1,000–2,000 times more effective, on a molar basis, as a primer than ApG. Since the distance from the 5' end of the β -globin mRNA to the first AG sequence is 46 nucleotides and the first AGC is 316 nucleotides there would be substantial intervening sequence between the 5' cap and the putative primer. The first and only AGCA in the globin mRNA is 547 nucleotides from the 5' end and is the longest sequence complementary to the virus 3' end. In order to achieve a capped cRNA molecule which has only 10–15 extra nucleotides beyond the 3' end of the template much of this intervening sequence would have to be removed and the 5' cap and associated sequences spliced

on to the primer sequence. This part of their model is highly speculative and exactly which sequences are transferred from the mRNA to the virus cRNA have not yet been determined.

Other capped eukaryotic mRNAs can act as primers *in vitro* but it is not yet clear whether *in vivo* infection with influenza virus stimulates a specific priming mRNA species or whether it uses the general pool of

existing mRNA molecules. If, as the authors propose, splicing does occur *in vitro* then influenza virus would provide a very valuable *in vitro* model for eukaryotic mRNA splicing. The virus transcriptase complex contains a nucleoprotein and three other proteins ranging in molecular weight from 85,000 to 95,000 daltons, any one of these are possible candidates for the proposed cap transfer and splicing activities. □

The Galactic map

from M. G. Edmunds

ONE disadvantage of living inside the Galaxy is that it is difficult to work out the details of its overall structure. The problems of determining the distance to sources of emission or absorption of radiation are acute, and we could speculate on how much easier it would have been if the Earth were situated in a globular star cluster well above the disk, allowing us to just look down and admire the grand design. But we are in the disk, and although some recent observations and theoretical models rather underline our ignorance, they do provide some interesting comparisons with observations of other galaxies.

As is well known, obscuration of light by interstellar dust particles limits the use of optical observations in the disk to at most a few kiloparsecs away, and it is the unobscured radiofrequency observations of interstellar gas which have yielded data over the distance of 10 kpc or so to the Galactic centre. It is fortunate that we do at least live fairly far out in the disk since, with the assumption of circular orbits about the centre, a little geometry will demonstrate that some information can be gained on the distribution of material which is closer to the centre than the Sun. Provided the angular velocity of the material about the centre decreases with increasing radius—which is reasonable provided total mass density decreases outwards—the maximum Doppler shift observed along a line-of-sight between 0° and 90° to the line joining the Sun and Galactic centre corresponds to material at the distance of that line-of-sight's closest approach to the Galactic centre. As long as there is emitting material present at this point, corresponding to the so-called 'terminal' velocity, then it is possible to map the circular rotation velocity as a function of radius to obtain a 'rotation curve'. The method works reason-

ably well between radii 4 and 9 kpc, but difficulties arise in interpreting the regions inside 4 kpc and outside 9 kpc. Near the centre the deviations from circular velocity (that is, inward or outward motions) can be as large as the circular motion itself, precluding reliable determination of a rotation curve or the inferring from velocities of the distance of material from the nucleus.

Outside the Sun's radius at 10 kpc, no terminal velocity occurs, except in the sense that the observation of material far out where the rotation was very, very slow would lead directly to a measurement of the Sun's rotational velocity about the centre of the Galaxy. This possibility has motivated a search by G. R. Knapp, S. D. Tremaine and J. E. Gunn (*Astron. J.* **83**, 1585; 1978) for emission from neutral atomic hydrogen along directions at about 90° to the Sun–Galactic centre line. The generally accepted value for the Sun's velocity is 250 km s⁻¹, although it must be admitted from the outset that this value could be wrong by 20%. If almost-stationary gas is observed far out in the Galaxy, its radial velocity relative to the Sun will therefore be about 250 km s⁻¹. However, at their sensitivity limits, Knapp *et al.* observe no emission with velocity greater than about 160 km s⁻¹, in agreement with previous searches. It has been suggested that this cut-off indicates an 'edge' to the Galactic disk, but Knapp *et al.* fit their observations with an interesting alternative model. What they do is assume that the density of gas in the disk (or rather the density of gas per unit surface area of the disk) falls off exponentially with radius—as is consistent with observations of hydrogen in external galaxies. Although the implied surface density of gas at 50 kpc out in the Galaxy is only about one hundredth of that measured in some large spirals, it is not unreasonable, since it is higher than extrapolation to this radius of measurements of the surface gas density in the nearby Andromeda spiral.

M. G. Edmunds is a lecturer in the Department of Applied Mathematics and Astronomy, University College, Cardiff.

They further assume that the rotational velocity falls off as a simple power of the radius, and adjust this radial dependence to obtain agreement between the observed 21-cm hydrogen line profiles and those predicted by their model. Their 'best fit' suggests a flat rotation curve further out than the Sun—that is, a circular velocity that is constant with radius.

Perhaps the flatness of the Galaxy's rotation curve should not be too much of a surprise, since studies of other spiral galaxies have shown similar constancy of velocity at a large radius. A prime example is a collection of 10 large spirals investigated by V. C. Rubin, W. K. Ford and N. Thonnard (*Astr. J. Lett.* **225**, L107; 1978) in which all the rotation curves found were still approximately flat out as far as they could be observed—which in one case was nearly 50 kpc from the nucleus. (This distance could be a factor of two smaller if a Hubble constant of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ were preferred to the authors' adopted $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$).

The implication of a flat rotation curve lies in the total deduced mass and size of a galaxy. If you are looking far enough out, then nearly all the mass is closer to the nucleus and the rotational velocity should have Keplerian form, that is, it should fall off as the reciprocal of the square root of the radius. If the velocity is constant with radius, then the further out you look the more mass you include. In approximate terms, if the rotation curve for our Galaxy is flat out to say 50 kpc (which is about the limit of the Knapp *et al.* hydrogen detection), then this implies perhaps five times as much mass in the Galaxy as would be implied by assuming Keplerian behaviour beyond 10 kpc. The Sun is no longer on the 'edge' of the Galaxy at all. It should be noted that this applies to the total mass, including stars—many of which will be faint, while the gas mass beyond 10 kpc is not particularly significant since, as mentioned above, its surface density is assumed to fall off exponentially. Although discounted by Knapp *et al.* it is not altogether clear how much their derived model would be altered should significant non-circular motions exist in the gas at large radii.

One requirement of this new model is that the rotational velocity of the Sun about the Galactic centre is reduced to 220 km s^{-1} . The old value of 250 km s^{-1} could be retained, but only with rather unpalatable assumptions. Either the disk might really cut off sharply at large radii—but the exponential model seems to fit rather well, or else the rotational velocities could still be increasing with radius at the Sun. This latter possibility is difficult to reconcile with well-established optical

observations, both of the direction-dependence of the velocity dispersion of stellar velocities in the solar neighbourhood and the nearby rotational motion as mapped by Cepheid variable stars whose distances can be estimated.

Recent neutral hydrogen observations also give further support to a revised picture of the inner regions of the Galaxy. For some years observations have been interpreted as implying that a systematic expansion of the inner regions is occurring. The cause of such non-circular motions has been attributed to a hypothetical (and rather energetic) explosion in the Galactic nucleus. It has been realised that the observed gas with remarkably circular velocities very close in to the nucleus is something of a mystery in this explanation, since considerable disruption could be inevitable if an explosion had occurred. R. J. Cohen and R. D. Davies (*Mon. Not. R. astr. Soc.* **186**, 453; 1979) suggest that the hydrogen observations can be equally well fitted by a non-explosive scheme. The model

accepts the non-circular motions as a normal state of affairs, and is a variant of a model proposed some years ago to account for the observed kinematics of molecular clouds near the Galactic centre. The model requires the existence of a suitably oriented bar-like structure near the centre, leading into spiral structure at about 1 kpc. The presence of a central bar is not unusual in external spiral galaxies, and indeed some workers would claim that the existence of such a bar is necessary to provide the driving mechanism which maintains spiral structure in galaxies without a gravitationally-perturbing companion. The expected gas flows along the bar would explain much of the observed non-circular motions.

Further theoretical modelling of the inner and outer regions of the disk will help to clarify the overall picture of the Galaxy, but it may be a long time before anything like a definitive map of our Galactic structure can be agreed. If only someone in Andromeda would send us a snapshot of the view. □



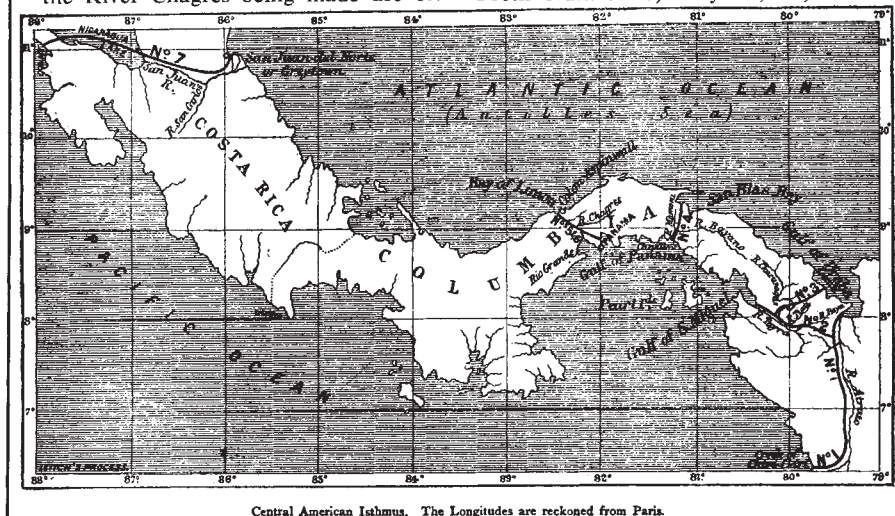
A hundred years ago

TO-DAY the long-talked-of International Congress on the subject of a canal across the Central American Isthmus meets in Paris under the presidency of M. De Lesseps. This question is a very old one, but the movement which has led up to the present Congress commenced only in 1875, at the instigation of Lieut. Lucien N. B. Wyse, of the French Navy. At the International Congress of Geography of that year the subject of the piercing of the American isthmus was seriously discussed...

... Nos. 5 and 6 are both in the Colon and Panama departments of Panama State, and, as will be seen, are to a considerable extent coincident. The former is 72 kilometres long, all the River Chagres being made use of.

The amount of excavation would be 57,000,000 cubic metres, and of embankment 5,000,000; there would be 25 sluices and no tunnel, and it would take six years to make. No. 6, again, would have no sluices, but tunnelling 6 kilometres long, with 47,000,000 cubic metres of excavation. It would be 75 kilometres long, and the rivers Chagres and Rio Grande would be utilised. Each would take about six years to make, and would cost about the same sum. They are near the Panama Railway, pass through a well-peopled region, and there is no difficulty as to ports. Lieut. Wyse's commission, however, advocated warmly No. 6 scheme, as being preferably to any other. The time wasted in passing locks, the difficulty and expense of maintaining them, and other considerations, induce them to advise that all idea of a canal with locks should be abandoned; and of all possible level canals with tunnels, that numbered 6 seems to this commission altogether the one presenting the most favourable conditions.

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Central American Isthmus. The Longitudes are reckoned from Paris.

review article

Cell-surface antigens induced by RNA tumour viruses

R. Kurth

Friedrich Miescher Laboratory, Max Planck Institute, Spemannstrasse 37-39, 74 Tübingen, FRG

E. M. Fenyö, E. Klein

Department of Tumor Biology, Karolinska Institute, Stockholm 60, Sweden

M. Essex

Department of Microbiology, Harvard University, School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115

Host animals show an immune response to the surface of cells infected with RNA tumour viruses. An element of this response is due to expression of viral structural antigens, but the major part is due to virus-induced cell-surface antigens (CSAs). This article compares the properties of CSAs of the avian, murine and feline retrovirus systems.

ONCOGENIC RNA tumour viruses (retroviruses) are widespread in nature. These viruses can be classified into groups according to their morphology or according to their natural hosts. As a general rule, viruses from evolutionarily related species share homologies in their single-stranded RNA genome, so their structural proteins are biochemically and antigenically related, but not identical (Fig. 1). Thus the horizontally transmissible RNA tumour viruses of chickens are distinct from the corresponding strains of mice and cats, which in turn are related.

Infection with RNA tumour viruses does not lead to cell lysis and it is an inherent advantage of this virus model that a number of different and stable virus-host cell interactions can be studied. Depending on whether leukaemia or sarcoma viruses are used, and whether cells from homologous (natural host) or heterologous (other hosts) species are used, the viruses will or will not replicate (Fig. 2) and may or may not cause malignant cell transformation. Lack of replication does not necessarily mean that the infecting viruses are lost, as they may become chromosomally integrated and some of their genes may still be transcribed and translated.

Some of the viral structural proteins (Fig. 1) may be expressed on the plasma membrane without any obvious impact on the growth of the infected cells. Since transformation changes the social behaviour of cells, in which the properties of the plasma membrane may play a crucial part, the question arises as to whether virus-transformed cells express additional proteins localised on the cell surface. Recent studies have shown that such tumour-associated, virus-induced cell surface antigens (CSAs) exist.

Early studies of oncornavirus-induced leukaemias and sarcomas revealed an immunological host response against these tumours. Whether this response is directed against viral structural proteins or CSA has been an open question.

In this article we review the information available on the immunologically unrelated CSAs of the avian, murine and feline oncornavirus systems to establish whether these antigens possess general and common characteristics. We ask four related questions: (1) Are the CSA virus proteins precursors or metabolites of the major virus structural proteins (fig. 1). (2) Are the CSA products of derepressed cellular genes. (3) Can CSAs act as transplantation-type antigens, that is, are they the major target antigens for a successful anti-tumour immune response by the host. And (4) are some CSAs related to the transforming proteins coded for by the RNA tumour viruses? If so, this could implicate the cell plasma membrane as the prime target organelle in malignant cell transformation and would indicate that cell-surface alterations may be responsible for the changed social behaviour of tumour cells.

We have summarised part of the vast amount of literature in this field in Table 1.

Definition of virus-related 'non-virion' CSAs

In the avian system, the sarcoma virus strains (ASV) infect, transform, and replicate in avian-derived fibroblasts. Avian lymphatic leukaemia viruses (ALV) infect and replicate in fibroblasts, but do not as a rule transform. All ASV strains tested

	<i>gag</i>	<i>pol</i>	<i>env</i>	<i>onc</i>	<i>c</i>	AA ... A3'
5'	Major structural core proteins	Polymerase RNase H DNA endonuclease	Envelope glycoproteins	Transforming protein(s)	Constant region	
Avian:	p19* p27 p12 p15	p90 p65 p32	gp85 gp37	pp60 ^{src}	?	
Murine:	p15 p12 p30 p10	p84	gp70 p15(E)	?	?	
Feline:	p15 p12 p30 p10	?	gp70 p15(E)	p65†	?	

Fig. 1 Schematic representation of RNA tumour virus genomes and their known gene products. Starting at the 5' end of the virion RNA, the first gene is *gag* (see ref. 101 for review). Its product is a polyprotein which undergoes post-translational cleavage to yield the major virus core proteins. The second gene is *pol*, coding for a pleiotropic enzyme with RNA-dependent DNA polymerase, RNase H and DNA endonuclease activity¹⁰²⁻¹⁰⁴. The third gene is *env* which codes for a precursor protein which is cleaved and glycosylated to yield virus envelope glycoproteins. The fourth gene *onc* codes for putative primary 'transforming proteins' whose presence in the cell lead to malignant transformation. The *onc* gene has so far only been localised for avian sarcoma viruses in which it is designated *src*⁵¹⁻⁵⁴. For brevity, intermediate protein processing steps have been excluded. No attempt has been made to illustrate individual genome lengths. *Molecular weight in thousands. Nomenclature according to ref. 105. †Candidate gene product. For details see refs 29, 42, 51-53.

to date, but no ALV, are able to induce a cross-reacting antigen designated tumour-specific cell surface antigen (TSSA); this suggests that TSSA expression is related to malignant fibroblast transformation¹⁻¹⁰. TSSA has been detected both by humoral and cellular immunity. Both antibodies and lymphoid cells from tumour regressor chickens, quails or mammals reacted with transformed cells^{1,11-15}. Similar immune parameters are demonstrable in animals hyperimmunised with ASV or with ASV-transformed fibroblasts. It seems unlikely such antigens are present on virus-induced lymphoma cells because even those ALV strains which can transform chicken fibroblasts do not induce the ASV-related TSSA^{10,16}.

In contrast to the avian system, in which antigenic differences between ALV- and ASV-infected fibroblasts were studied (see refs 17-19 for reviews), the mouse system involves serological

characterisation of lymphoma cells. In the mouse, the Moloney (M-MuLV) or the Gross (G-MuLV) leukaemia virus strains are most extensively analysed. Sera of mice immunised with syngeneic lymphoma cells^{20,21} react with viable Moloney lymphoma cells (via the Moloney cell surface antigen, MCSA) and the response against the lymphoma cells is unrelated to the virus neutralising immune response. Antisera against the Gross cell surface antigen (GCSA) were usually produced across the histocompatibility barrier because in this way the response was stronger. Alloantibodies did not enter the reaction since the target cells were syngeneic with the serum donor²².

After regression of tumours caused by feline sarcoma virus (FeSV), the serum of cats contains antibodies reactive with viable lymphoma cells²³⁻²⁵. The antigen detected in this way has been designated feline oncornavirus-associated cell membrane

Table 1 Cell-surface antigens induced by avian, murine and feline oncornaviruses

Property	Avian (TSSA)	Murine		Feline (FOCMA)	TSSA	References		FOCMA
		(MCSA)	(GCSA)			MCSA	GCSA	
(1) Usual method(s) of detection	MIF* CMC	MIF AMC	MIF AMC	MIF AMC	1, 30, 91	20, 21	22	24, 39, 99
(2) Expression on:								
(a) Non-producer sarcoma virus transformed cells of homologous species	+	-	NT	NT	6	68, 71	—	—
(b) Non-producer sarcoma virus transformed cells of heterologous species	+	-	NT	+	6, 7, 15, 30, 31	69	—	29, 39, 72
(c) Producer sarcoma virus transformed cells of either homologous or heterologous species	+	+	NT	+	1, 2, 30, 31	69	—	29, 39, 72
(d) Leukaemia virus infected non-transformed fibroblasts	-	+	+	-	1, 2	106	107	29, 39, 72
(e) Lymphoma biopsies and cultures from induced or spontaneous virus tumours	NT	+	+	†	—	85	22	73-75
(f) Normal lymphoid cells	NT	-	+	-	—	63	107	73-75
(3) Distinct from virus structural proteins	+	+	-	+	1, 2, 6, 91, 92	21, 36, 37	34, 35	29, 39, 40, 42, 74
(4) Included into the virion								
(a) Leukaemia viruses	-	-	+	-	15	36	34	29, 40
(b) Sarcoma viruses	NT	NT	NT	+	—	—	—	29, 40
(5) Virus specificity	Group	Subgroup	Subgroup	Group	1, 2, 7, 12, 15	60	107	74, 76
(6) Distinct from embryonic antigens	Yes	NT	NT	NT	5, 14, 15	—	—	—
(7) Approximate MW	NT	180-192,000 92,000 52,000	180,000 85,000 45,000	65,000	—	36	34	29, 42
(8) Glycosylated	NT	Yes	Yes	No	—	37	34	110
(9) Relevance for anti-tumour immunity <i>in vivo</i>	+	+	+	+	10, 13, 93, 94	58, 59, 85, 95	107-109	24, 76, 96, 99

MIF, membrane immunofluorescence; CMC, cell-mediated cytotoxicity; AMC, antibody mediated cytotoxicity; NT, not tested.

* Similar results were obtained regardless of which method had been used.

† Both virus producer and non-producer lymphoma cells are positive for FOCMA.

antigen, or FOCMA²⁵. FOCMA is expressed on FeSV transformed fibroblasts as well as on feline leukaemia virus (FeLV) transformed lymphoma cells.

In some other C-type virus models²⁶⁻²⁸ similar CSAs could not be demonstrated and the host immune response seemed to be directed against viral structural proteins only. Similar situations may be encountered even in the known CSA-positive systems, depending on several factors. To give some examples: (1) The observation that FOCMA expression is much lower on FeSV-transformed rat cells as compared to similarly transformed mink or monkey cells²⁹ makes the choice of target cell important. (2) In the avian system, mouse sera react better with ASV-transformed syngeneic mouse cells than with ASV-transformed chicken fibroblasts^{30,31}. (3) Also in the avian system, it seems that predominantly cell-mediated immunity rather than humoral immunity is induced against TSSA in chickens and quail^{1,11,12,15}, explaining why it is difficult to produce high titre

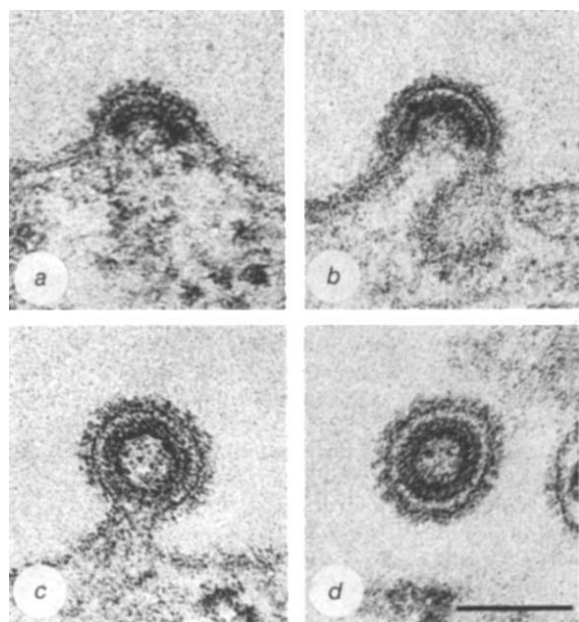


Fig. 2 Sequential stages of the budding process of replicating oncornaviruses (a-d). Virus: the Friend strain of murine leukaemia viruses. Scale bar, 100 nm. Electron micrographs were provided by Dr H. Frank.

anti-TSSA antisera. (4) There may also be genetically determined differences in antigen recognition, as illustrated by the A/Sn and the BALB/c mouse strains which develop only a weak immune response against MCSA³².

Isolation and characterisation of CSA

Avian TSSA was initially isolated by immunoprecipitation with antisera of chickens immunised with ASV. The molecule was a glycoprotein of MW 100,000 (gp100)³³. In subsequent experiments in which additional antisera were used, the same gp100 was also found on ALV-infected non-transformed cells, although in smaller amounts. Thus the expression of gp100 is not specific for malignant transformation by ASV. gp100 may instead represent a cell-surface precursor protein for the virus envelope glycoprotein gp85 (refs 6, 10).

MCSA and GCSA were isolated from lymphoma cells by related but slightly different approaches. GCSA was isolated by immunoprecipitation followed by polyacrylamide gel electrophoresis^{34,35}; whereas with MCSA, antigenic proteins were detected by cytotoxicity inhibition subsequent to electro-

phoresis and elution from gel slices³⁶. The results were similar in that both MCSA and GCSA specificity were detected on several molecular species of MWs 150,000-193,000, 85,000-92,000 and 45,000-52,000.

The GCSA-positive proteins also carried antigens of *gag*-gene products (see Fig. 1 for localisation of *gag* gene in the viral genome), as they were precipitable with *gag*-specific antisera³⁴. These molecular species also bound to anti-p30 immunoadsorbents³². In contrast, MCSA-positive molecules did not react with antisera specific for virion structural proteins^{36,37} nor did they bind to anti-p30 columns³⁸. This indicates that molecules carrying MCSA specificity are distinct from virion proteins.

Another approach, however, indicated that on the surface of viable lymphoma cells, MCSA may be physically linked to *gag* proteins. In co-capping experiments MCSA, p30 and p12 were found to be associated³⁸. The apparent discrepancy between co-capping and precipitation results may indicate that the physical linkage between MCSA and p30 is disrupted during solubilisation. Alternatively, co-capping may not necessarily be proof for association of antigens on the same molecule. In addition, part of MCSA is linked to p15, the 5'-terminal *gag* protein, because 10-15% of MCSA was bound to anti-p15 immunoadsorbents.

For isolation and characterisation of FOCMA, cloned lines of FeSV-transformed non-producer mink cells were used^{29,39,40}. These cells were negative for gp70 and p30 but expressed the 5'-terminal *gag* proteins p15 and p12. On agarose gel filtration, a protein of MW ~85,000 was isolated which contained p15 as well as p12 and showed FOCMA specificity since it inhibited the anti-FOCMA activity of cat sera. Pulse-chase experiments with ³⁵S-methionine established that this protein is cleaved into two smaller components, one of MW ~65,000 carrying only the FOCMA specificity and another of MW ~25,000 containing p15 and p12²⁹. This result was confirmed by rescue of FeSV from transformed non-producer cells with an unrelated oncornavirus strain^{40,41}. The same 85,000 MW protein containing p15, p12 and FOCMA was found in the virion pseudotypes. This protein was not present in FeLV particles^{28,40,41} possibly because cleavage occurs in the presence of FeLV, and unless FOCMA is covalently linked to the virion structural proteins, as in the p85, it is not incorporated into the virion.

Using immunoprecipitation and polyacrylamide gel electrophoresis, FOCMA has been identified on both FeLV producer and non-producer lymphoma cells as a molecule of MW ~70,000⁴². This antigen does not react with antisera to the virus structural proteins. The p70 appears similar to the lower MW FOCMA molecule (p65) found on FeSV-transformed non-producer cells.

It thus seems that the three antigens, FOCMA, GCSA and MCSA, are present on multiple molecular species and at some stage they may be linked to *gag* (precursor) proteins. The solubilised GCSA is probably identical to a precursor polypeptide carrying several specificities. FOCMA is present on a *gag* precursor molecule, but is cleaved off as a distinct entity. FOCMA is also expressed on non-producer lymphoma and sarcoma cells in the absence of *gag* and *env* proteins. A small fraction of MCSA is also linked to the *gag* p15 in producer lymphoma cells. Thus FOCMA and MCSA show close similarities in that they are both linked to the 5'-end protein(s) and represent distinct antigenic specificities.

In another group of RNA tumour viruses, the acute leukaemia viruses of chickens and mice, virus-strain specific polypeptides are synthesised on infection⁴³⁻⁴⁹. These polypeptides, similar to FOCMA and MCSA, carry antigens linked to part of the *gag* proteins near the 5'-end of the viral genome. Their continued presence in the transformed cell is probably necessary to maintain the transformed state of the cell, as was first indicated with a temperature-sensitive mutant of avian erythroblastosis virus⁵⁰. Thus, the polypeptides may also function as transforming proteins. It is not yet known whether these polypeptides, or their metabolites, appear on the cell surface of acute leukaemia virus-transformed cells. (See note added in proof.)

Genetic determination of the CSA and relationship to malignant transformation

Once it is established that CSAs are not identical with virion proteins, it is logical to ask whether they are encoded by the virus. Supporting evidence is only indirect, and it is different for each system.

In the avian system, the TSSA on tumours and *in vitro* transformed fibroblasts cross-react, regardless of the inducing ASV strain, and are thus group-specific^{7,17-19}. Because cells of many different species have been shown to exhibit TSSA, it is probably coded for by the virus. Genetic studies indicate that TSSA may be related to malignant transformation and the synthesis of a functional *scr* gene product (Fig. 1)⁵¹⁻⁵⁴, because with temperature-sensitive and deletion mutants of ASV the transformed cellular phenotype correlates with TSSA expression⁵⁵⁻⁵⁸.

Erikson and co-workers have shown the *scr* gene product of ASV to be a 60,000 MW phosphoprotein (pp60^{src}, Fig. 1)⁵¹⁻⁵³. pp60^{src} is located predominantly in the cytoplasm⁵², but may be synthesised as a 62,000 MW precursor containing a 2,000 MW amino-terminal hydrophobic signal sequence characteristic of secretory and integral membrane proteins⁵⁴. This indicates that pp60^{src} may be sequestered in the plasma membrane, but a direct immunological relationship between pp60^{src} and TSSA has not yet been demonstrated using sera specific for pp60^{src} or TSSA (A. Huesgen, T. Tanaka and R. K., unpublished). The possibility remains, however, that a proteolytically processed and possibly glycosylated metabolite of pp60^{src} with altered antigenicity may appear on the cell surface.

In the mouse, Moloney lymphoma sublines^{59,60} expressing low amounts of MCSA have been immuno-selected. These sublines produce virus particles deficient in MCSA-inducing capacity⁶¹⁻⁶³. This suggests that MCSA is virally determined. Thus, selection for a cellular phenotype (expression of MCSA) produces a virus variant that imposes the same phenotypic property.

The observation that MCSA is regularly present on Moloney lymphomas but absent from lymphoid cells of normal mice (even if they express viral structural protein antigens corresponding to gp70 and p30 of an endogenous C-type virus) suggests that MCSA may be related to transformation. Recent studies showed that in M-MuLV-inoculated viraemic mice the target tissue for M-MuLV leukaemogenesis, the thymus, is the first organ to express MCSA⁵⁴. MCSA-positive cells in organs other than thymus, for example, in lymph nodes or spleen, occur only in overt leukaemia. MCSA-positive thymus cells, however, are non-malignant, as they do not grow as progressive tumours in syngeneic recipients. Thus, the presence of MCSA on the cell surface does not strictly indicate malignant transformation, but is associated with viral gene amplification preceding leukaemia development. This is reminiscent of the amplification phenomenon, that is, a high level of viral protein expression in the pre-leukaemic thymus of AKR mice^{65,66} and of mice with germ-line integrated M-MuLV⁶⁷.

Whereas it seems that M-MuLV codes for MCSA, M-MuSV-transformed sarcoma virus-positive, leukaemia virus-negative (S+L-) cells of homologous (NIH Swiss and BALB/c)⁶⁸ or heterologous (mink) species do not express MCSA⁶⁹. Aoki *et al.*⁷⁰ obtained slight antigenic differences between MuSV transformed and non-transformed 3T3 cells using immunoelectron microscopy, but this antigen(s) did not function as a transplantation antigen⁷¹.

After superinfection with MuLV, S+L- cells expressed MCSA⁶⁹. The rescued genetic information coded for MCSA induction, because after rescue with the MCSA-induction-defective M-MuLV variant, MCSA appeared on the cells and the virus produced had MCSA-inducing capacity⁶².

FOCMA is probably coded for by the viral genome, as it is present on FeSV-transformed fibroblasts of both feline or non-feline species, such as dog and mink^{29,38,72}. It is also expressed on lymphoid tumour cells⁷³⁻⁷⁵. It is absent from normal lymphoid

cells and fibroblasts, even from those infected with (but not transformed by) FeLV^{38,72-75}. In contrast to the murine system, but like avian TSSA, FOCMA is present on cells non-productively transformed by FeSV^{29,38,72}. It seems to be tumour- and/or transformation-specific for both FeLV and FeSV.

All strains of FeLV and FeSV so far checked can induce FOCMA *in vivo*^{23,76,77}. Lymphoid tumour biopsies express the antigen whether they are T, B or null cells^{73,75}. When thymic lymphomas are induced, FOCMA-positive lymphoid cells can be found in the thoracic cavity a few weeks before clinical leukaemia is present⁷⁸.

Thus on the basis of the available data, TSSA, MCSA and FOCMA all seem to be virus coded. An alternative possibility remains—that they are products of cell regulatory gene(s) de-repressed concomitant to malignant transformation.

Relevance of CSA for oncogenesis and graft rejection

Study of tumour antigens must depend on antigens defined by the immunological reactivity of the tumour host species. Consequently, it may be envisaged that these antigens are involved in graft rejection and also in the host response during oncogenesis.

Chickens⁷⁹⁻⁸¹, mice⁸² or cats^{83,84} neonatally infected with oncornaviruses become viraemic, develop low or no anti-tumour immunity and usually succumb to their tumours. In contrast, infected adults usually resist both persistent viraemia and tumour development but respond with antibodies^{20,24} and cellular immunity⁸⁵.

Lymphocytes and antibodies that react against virus-envelope antigens may have beneficial immunological effects against oncogenesis because they reduce viraemia and the number of secondary infections. Moreover, such antibodies may kill the virus-infected cells due to the virus envelope antigens expressed in the plasma membrane^{1,2,86}. It has been shown in mice and cats that passive immunity achieved by administration of purified anti-gp70 immunoglobulins prevents permanent infection with leukaemogenic viruses and therefore development of certain diseases⁸⁷⁻⁸⁹. Whereas antibodies to the viral envelope proteins limit virus spread, immunity to CSA results in the death of tumour cells, regardless of the presence or absence of virus structural proteins⁹⁰.

In mammals, non-productive ASV-transformed cell lines express TSSA^{30,31,91,92} and do not have viral proteins on the cell surface. Mice and rats can be protected from oncogenesis by preimmunisation with syngeneic ASV-transformed cells^{93,94}, indicating that TSSA can serve as an efficient transplantation-type antigen in the immune response.

Several findings suggest that MCSA plays an important part in the host response against lymphoma transplants. First, when a large number of Moloney lymphomas were compared, *in vivo* rejectability correlated with serologically detectable MCSA expression⁸⁵. Second, in a group of back-cross mice segregating for low- and high-titre anti-MCSA response only those with antibodies to MCSA were protected against lymphoma transplants⁹⁵. There was no correlation between successful tumour rejection and the anti-viral response. Third, selection of cells for lack of sensitivity to anti-MCSA sera led to immunoresistant sublines⁵⁹ with unaltered expression of virion protein antigens⁶⁰.

In the feline system, antibody response to FOCMA is correlated with the clinical status of the animals⁹⁶⁻⁹⁸. It is conceivable that prevention of tumour development is—at least partially—mediated by lytic complement-dependent antibody, which in fact, functions best in the presence of cat complement. Since the tumour cell is usually a T cell, it may be that evolutionary selection favoured an immunological mechanism different from classical T-cell mediated immunity^{99,100}. Cats with virus-producer tumours sometimes have only temporary regression of tumours when given anti-gp70 serum. If subsequent relapses then occur in the absence of FOCMA immunity, the surviving tumour cells appear to have undergone

immunoselection since they no longer express gp70 but have retained FOCMA⁸⁹. Antibodies to FOCMA are effective in immunotherapy for both leukaemia and sarcoma^{87,90}.

Conclusions

Host species respond with immunity against the cell surface of RNA tumour virus-infected cells. Part of this reactivity is due to expression of some of the viral structural antigens, but although antiviral immunity may abrogate infection, resistance against tumours is mainly due to the responses against the CSAs. Recognition of antigens is influenced by host genetics and may show wide variations.

GCSA cannot be distinguished from viral (gag) precursor proteins. Part of the MCSA and FOCMA reactive molecules also contain the antigenic determinants of viral 5' terminal gag protein(s), which, in the case of FOCMA, were shown to be cleaved off as distinct entities. Thus these CSAs all correspond to precursors of the viral gag gene, some also containing additional as yet undefined entities (here referred to as MCSA and FOCMA).

The relationship of CSA to transformation seems to be different in the various systems. FOCMA is specific for trans-

formation by both FeLV and FeSV. TSSA is specific for transformation of fibroblasts with ASV, but is probably not expressed on ALV transformed lymphoma cells. On the other hand, MCSA is similar to GCSA in that it is expressed on preleukaemic non-malignant thymus cells.

Finally, it is not yet clear whether the expression of functional TSSA and FOCMA is sufficient to establish the malignant character of correspondingly infected cells. This question could be answered if, for example, oncogenic tumour virus mutants defective in antigen-induction become available.

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Note added in proof: D. Baltimore and O. N. Witte recently obtained evidence which suggests that the cytoplasmic Abelson MuLV polyprotein⁴⁷ which is transformation related is also expressed on the cell membrane (personal communication).

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articles

Geomagnetic variation during the late Pleistocene period and changes in the radiocarbon time scale

Mike Barbetti

Physics Department, University of Adelaide, GPO Box 498, Adelaide 5001, Australia

Kevin Flude

57 Pratt Street, Camden Town, London NW1, UK

The Earth's magnetic field appears to have been weaker than it is today for much of the period 50,000–10,000 yr ago. The geomagnetic record can be used to predict changes in the production of radioactive nuclides by cosmic rays interacting with the atmosphere.

EVIDENCE has been accumulating for dramatic changes in the Earth's magnetic field during the late Pleistocene. Polarity events or excursions (deviations $\geq 40^\circ$ in the field direction) have been found in rocks, sediments and ancient fireplaces of various ages in many parts of the world. Some of the records, particularly those from sediments, may be less reliable than others but there can be little doubt that at least one large excursion has occurred during the past 50,000 yr. Considerably less information is available about changes in geomagnetic strength. We present here a new result and review other data already published. A broad outline of the changes is beginning to emerge and, with it, further clues to the nature of geomagnetism and the origin of excursions. We also discuss the effects that these changes might have had on the production of radioactive nuclides, especially ^{14}C , in the atmosphere. Some of the charged particle flux from interstellar space and the Sun is deflected by the Earth's magnetic field, and is thereby prevented from interacting with the atmosphere. The observed variations in geomagnetic strength are bound to have strongly modulated that flux during the late Pleistocene. We make some predictions about the ^{14}C time scale and compare them with independent evidence from other dating methods.

A new estimate of geomagnetic strength from Australia

We have investigated an ancient fireplace at Lake Mungo in southeastern Australia (F51, latitude 33.7°S , longitude 143.1°E). Stratigraphic details and the ^{14}C age determination of $19,420 \pm 360$ yr b.p. (ANU-668), from charcoal preserved within the fireplace, have been described previously⁴. The thermoluminescent age is $16,900 \pm 5,000$ yr BP. (Huxtable and Aitken, personal communication). Measurements of remanent magnetisation on a few specimens of underlying partially-baked sediment gave directions very close to that expected from an axial dipole.

Measurements of ancient field strength were made on oven-stone specimens according to the modified Thelliers' tech-

nique^{14–16} following our normal laboratory procedures⁹. The results are summarised in Table 1 and examples are shown in Fig. 1. The specimens exhibited varying degrees of non-ideal behaviour, but consistent estimates of field strength were obtained after some of the higher-temperature measurements were excluded from the final analyses (Fig. 1). We attribute the non-ideal behaviour to incomplete heating in antiquity³ or subsequent weathering of the baked sediment^{18,19}, and note that much lower estimates would have been obtained for those specimens showing large deviations if the higher-temperature points had been included.

The site mean estimate for ancient field strength is given in Table 1, and a corresponding value for dipole moment is included in Table 2.

Geomagnetic strength estimates for the late Pleistocene

Table 2 summarises all well dated results known to us at present. Radiocarbon and thermoluminescent ages are listed separately, and virtual geomagnetic pole (VGP, ref. 20) positions are given when available. As field strength varies with latitude we have estimated the ancient geomagnetic dipole moment according to one or the other of two possible schemes.

For sites where the ancient geomagnetic direction is known with reasonable precision we have calculated a virtual dipole moment (VDM, ref. 13), defined as the strength of an imaginary dipole source at the Earth's centre which, if inclined at an angle to the rotational axis to produce the observed direction, would yield the observed ancient field strength at the site. The 95% confidence limits include contributions from the uncertainties in both geomagnetic direction and strength; the latter was obtained by multiplying the standard error of the mean by the appropriate value of Student's t test.

For sites where the ancient geomagnetic direction is known either poorly or not at all, we have calculated a virtual axial dipole moment (VADM), which differs from a VDM only in that the source is constrained to be coaxial with the Earth's rotational axis. Two values for the 95% confidence limits are then given; the first corresponds to the uncertainty in field strength alone, and the second includes a contribution derived from an assumed uncertainty of 20° in the field direction. We use the wider confidence limit elsewhere in this article.

The data are shown in Fig. 2, and it can be seen that many of the estimates are lower than the present-day dipole moment of

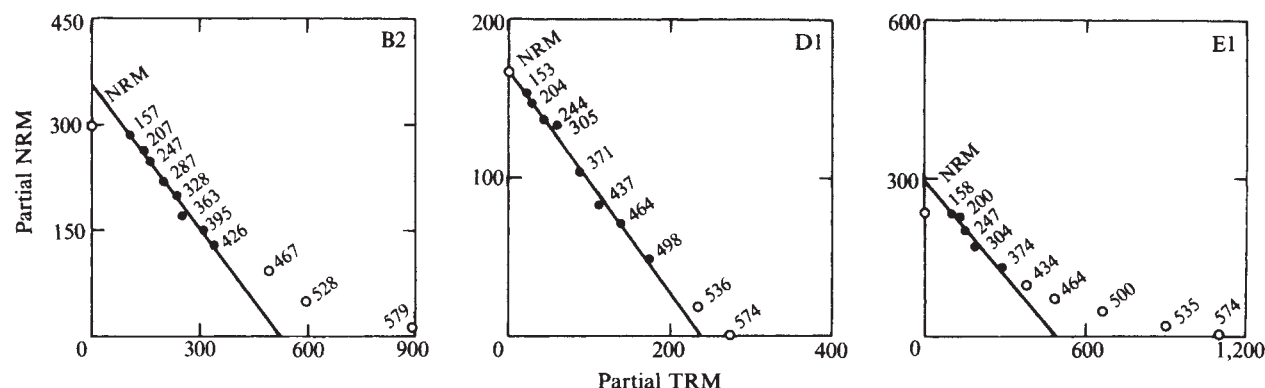


Fig. 1 Representative ancient field strength determinations for fireplace F51. The partial natural remanent magnetisation (NRM) which remains in the specimen after laboratory heating and cooling in zero magnetic field is plotted against the partial thermoremanent magnetisation (TRM) acquired by the specimen in a second heating and cooling cycle in a known laboratory magnetic field ($\sim 49 \mu\text{T}$ for these specimens). The units are $\mu\text{A m}^2 \text{ kg}^{-1}$, the horizontal scales are twice the vertical ones, and numbers near the points give the laboratory heating temperatures in $^{\circ}\text{C}$. Lines have been fitted to the sets of points represented by solid symbols, using the maximum likelihood method¹⁷ and rejection criteria described previously⁹. The slopes of the lines are measures of the ratio of NRM to TRM, and estimates of ancient field strength (Table 1) are derived on the basis that NRM and TRM are proportional to the magnetic field in which they were acquired.

$8 \times 10^{22} \text{ A m}^2$ (and the average Pliocene–Pleistocene value of $8.9 \times 10^{22} \text{ A m}^2$; ref. 21). The exceptions are the high values associated with the Lake Mungo geomagnetic polarity excursion and the three data between 12,000 and 10,000 yr b.p..

Implications for geomagnetism and the origin of excursions

Although there are still substantial gaps in the record (Fig. 2), the style of geomagnetic variation seems to be different from the familiar quasi-sinusoidal variation observed in Holocene times²⁶. We suggest that the apparent Holocene periodicity of $\sim 8,000$ yr may not be a fundamental one for the geomagnetic dynamo.

The late Pleistocene seems to be characterised by low values of dipole moment, except for a brief period during the Lake Mungo polarity excursion. That seems, at first, to contradict what one might expect to find by analogy with the behaviour observed during older polarity reversals, in which low field strengths are generally associated with anomalous directions and normal field strengths are found on either side of the transition region. However, low field strengths may sometimes persist for several thousand years before and after a reversal²⁷, and there are two records of a sudden large increase in strength at the centre of a polarity transition²⁸. The late Pleistocene record does not, therefore, seem abnormal.

The apparent absence of anomalous behaviour in some parts of the world at times when excursions are observed in other places, and the high field strengths associated with the Lake Mungo excursion, have led to the suggestion^{6,29–31} that sources located near the outer boundary of the Earth's core may be involved. If so, excursions, like polarity reversals, might be more likely to occur when the dipole field has been depressed for several thousand years; that could even be a necessary condition for reversals or excursions to occur. The sources responsible might be similar to those which produce the non-dipole part of the present-day field, and enhancement on some occasions might explain the high field strengths which have sometimes been observed.

Note that TL dates for the Laschamp 'event' are similar to those for the Lake Mungo excursion (refs 7, 22, and Fig. 2b) and that a double excursion was recorded at about the same time in sediments at Mono Lake, California³². Excursions have also been recorded in deep-sea sediments of about the same age^{33,34}. Unfortunately, the precision of most of the dates is not yet good enough to determine whether excursions in different parts of the world are simultaneous or separated by a few thousand years.

That, together with the scarcity of geomagnetic strength determinations, makes it difficult to be more precise about the origin of those excursions.

There is evidence to suggest that 'excursions' (refs 23, 24) in Swedish sediments dating from around 13,000 to 12,000 yr BP (the proposed Gothenburg excursion) may reflect sedimentation processes and not geomagnetic behaviour²⁵. That conclusion is supported by the fact that normal geomagnetic directions and strengths have been found in three ancient hearths in France which are dated at $12,000 \pm 500$ yr b.p. (Table 2 and Fig. 2).

Changes in the radiocarbon time-scale

We have attempted to use the geomagnetic record (Fig. 2) to estimate probable trends in the ^{14}C time-scale during the late Pleistocene. Our predictions assume that no significant long-term changes have occurred in the galactic cosmic ray flux, the flux of energetic particles from the Sun, the interplanetary magnetic field and the various terrestrial carbon reservoirs. We used the Lingenfelter and Ramaty³⁵ relationship to estimate the production rate of radiocarbon in the past.

The results are shown in Fig. 3, where they may be compared with independent evidence from a growing number of comparisons between ^{14}C and other dating methods.

If the dipole moment was constant for a long period beforehand and the value was within the confidence limits observed at $\sim 50,000$ yr BP (Table 2 and Fig. 2), then radiocarbon dates at

Table 1 Palaeomagnetic field strengths from fireplace F51

Specimen	Temperature range* ($^{\circ}\text{C}$) (n points)	Ancient field strength† (μT)
B1	158–428 (8)	23 ± 2
B2	157–426 (8)	33 ± 4
C	146–381 (8)	27 ± 3
D1	153–498 (8)	34 ± 4
E1	158–374 (5)	29 ± 14
F	146–710 (12)	27 ± 10
		Mean 29 ± 4

Specimens with the same letter were cut from the same ovenstone; specimens identified by letter only were small fragments found during excavation, and were not cut into cubes for measurement.

* Temperature range and number of points used in the final line-fitting analyses on the NRM–TRM diagrams.

† Estimates of ancient field strength are given with 95% confidence limits.

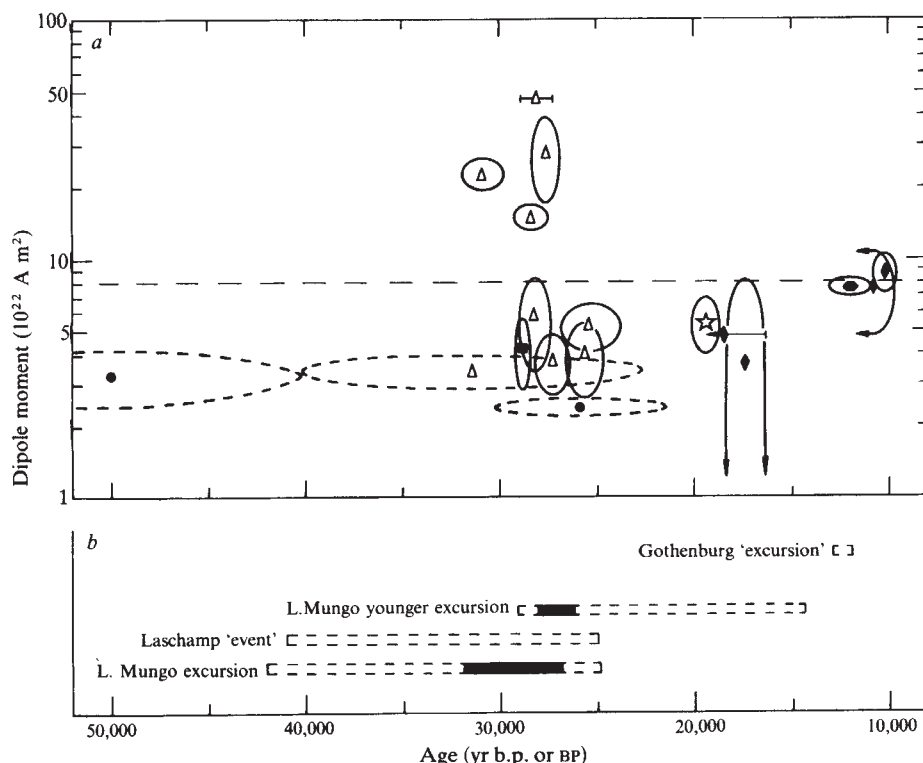


Fig. 2 a, Dipole moment estimates for the period 50,000–10,000 yr ago. Solid (open) symbols identify data from sites in the Northern (Southern) Hemisphere according to the key in Table 2, and surrounding ellipses indicate 95% confidence limits for moments and 2s limits for ages. Dipole moments are plotted on a logarithmic scale, and the dashed horizontal line marks the present-day dipole moment of $8 \times 10^{22} \text{ A m}^2$. Conventional ^{14}C ages, when available, have been used in preference to TL ages because of their greater precision and in those cases the ellipses are continuous; TL dates with their much broader 2s limits have been used in three cases identified by dashed ellipses. b, Age ranges (2s limits) for some of the geomagnetic polarity excursions proposed for the late Pleistocene. Solid lines indicate ^{14}C ages, and dashed lines TL or varve ages. High values of dipole moment are associated with the Lake Mungo excursion, and low values with what may possibly be a younger excursion at Lake Mungo. Age ranges for the Laschamp 'event' (TL, 2s; ref. 22) and the proposed Gothenburg excursion (varve; refs 23–25) are shown for comparison.

the end of that period would be somewhere between 4,000 and 5,500 yr too young. If, on the other hand, the dipole moment was higher than the Plio–Pleistocene average value before 50,000 yr BP, radiocarbon dates would be approximately equal to absolute dates. We therefore expect radiocarbon dates to be

between zero and 5,500 yr too young. There are no geomagnetic strength measurements between 50,000 BP and 31,000 yr b.p., but comparative $^{230}\text{Th}/^{234}\text{U}$ ages suggest that ^{14}C ages are again too young at ~40,000 yr BP and perhaps less so at ~32,000 yr BP.

Table 2 Summary of well dated dipole moment estimates for the late Pleistocene period

Site	^{14}C (yr b.p.)	Age TL (yr BP)	Ref.	Palaeomagnetic results		Ref.	Symbol
				VGP (°N, °E)	Dipole moment (10^{22} A m^2)		
Maniana Pali I, Hawaii	10,140 ± 300	*	1	63 265	8.7 ± 1.5	2	◆
Maniana Pali II, Hawaii	>10,140 ± 300	—	1	70 45	7.6 ± 2.9	2	◆
Etiolles/Marsangy, France	12,000 ± 500	—	3	84 106	7.6 ± 0.7	3	●
Hilina Pali I, Hawaii	17,360 ± 650	*	1	73 357	3.7 ± 4.4	2	◆
Hilina Pali II, Hawaii	>17,360 ± 650	—	1	?	4.8§	2	◆
Lake Mungo (F51), Australia	19,420 ± 360	$16,900 \pm 5,000^\dagger$	4	~n.g.p.‡	$5.4 \pm 0.8 (\pm 1.4)§$	This work	☆
Lake Mungo (F20), Australia	25,310 ± 810	—	5	~n.g.p.	$5.3 \pm 0.2 (\pm 1.2)§$	6	△
Lake Mungo (F17), Australia	25,570 ± 520	—	4	~n.g.p.	$4.0 \pm 1.1 (\pm 1.4)§$	6	△
Lake Mungo (F6), Australia	27,270 ± 470	21,800 ± 3,700	4, 7	3 238	3.8 ± 1.1	6	△
Lake Mungo (F16), Australia	28,140 ± 370	—	4	~n.g.p.	$5.8 \pm 2.1 (\pm 2.4)§$	6	△
Royat, France	—	25,800 ± 2,200	8	47 303	2.4 ± 0.2	9	●
Lake Mungo (F14), Australia	—	31,400 ± 4,400	7	−48 338	3.4 ± 0.5	6	△
Dolní Věstonice, Czechoslovakia	28,800 ± 170	33,000 ± 3,000	10	?	$4.2 \pm 0.7 (\pm 1.4)¶$	11	■
Lake Mungo (F9), Australia	27,530 ± 340	—	—	−8 238	$28.1 \pm 11.0¶$	6	△
Lake Mungo (F12), Australia	28,000 ± 410	—	—	−6 47	47.2	6	△
Lake Mungo (F8), Australia	28,310 ± 410	33,500 ± 4,300	4, 7	13 230	15.1 ± 2.1	6	△
Lake Mungo (F7), Australia	30,780 ± 520	—	—	−28 34	$22.8 \pm 3.2¶$	6	△
Boissejour, France	—	~50,000	8	?	$3.3 \pm 0.2 (\pm 0.9)§$	9	●

Sites are listed approximately in order of age. Conventional radiocarbon (^{14}C) ages are expressed in yr b.p. (before 1950 AD); they are based on a 5,568-yr half life and are not corrected for possible variations in atmospheric ^{14}C content. Thermoluminescent (TL) ages are considered to be absolute ages, and are expressed in yr BP. All age uncertainties are equivalent to one standard error (1s). Virtual geomagnetic pole (VGP) positions are listed when data is provided in the original reference. Dipole moments are virtual dipole moments (VDM, ref. 13) unless otherwise indicated. Confidence limits (95%) include contributions from uncertainties in both field direction and strength (s.e.m. multiplied by the appropriate value of Student's *t* test, provided results are available for at least three specimens). Virtual axial dipole moments (VADM, see text) are given for sites with few or poor-quality data for ancient geomagnetic direction, and in those cases two sets of 95% confidence limits are given; the first corresponds to the uncertainty in ancient field strength alone and the second (in brackets) includes a contribution from the uncertainty in geomagnetic direction (assumed to be 20°). Symbols in the last column are those used in Fig. 2.

* Thermoluminescence studies have been made but no dates are available¹².

† Unpublished date (archaeological dose, 2.70 krad; dose-rate = 0.16 rad yr^{−1}; Huxtable and Aitken, personal communication (1979)).

‡ Near the north geographic pole, for sites with few or poor-quality data; ? signifies no data available.

§ Virtual axial dipole moment.

¶ Virtual axial dipole moment, calculated from ancient/present field ratio in the reference with present field taken as 48 μT.

‖ Ancient field strength estimates from ovenstone and baked sediment specimens in the reference have been averaged.

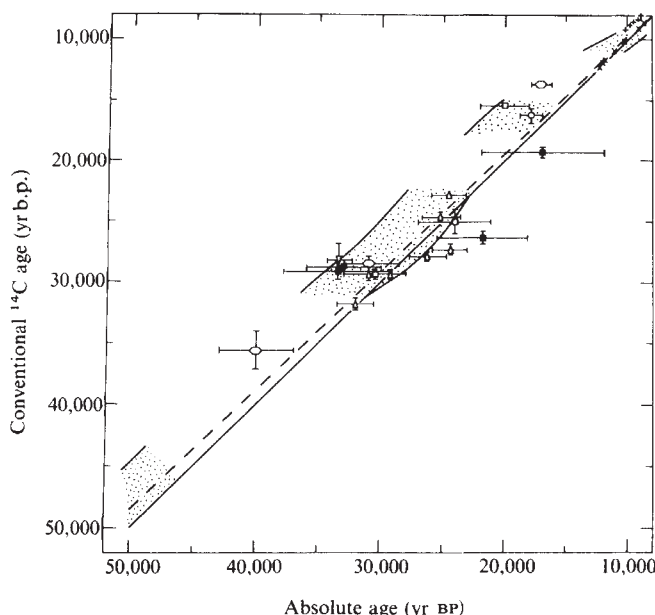


Fig. 3 Relationship between ^{14}C and absolute ages for the late Pleistocene. The solid 45° line indicates the ideal relationship, and the dashed line the relationship expected because ^{14}C dates are calculated using a 5,568-yr half life (which is known to be about 2.8% too small). Deviations from the dashed line reflect variations in the ancient atmospheric concentration of ^{14}C . Points with standard errors are derived from published comparisons of ^{14}C ages with those obtained by other methods; viz., TL dates from ref. 10 (●), ref. 7 and personal communication (■); varve ages from ref. 40 (+) and ref. 41 (×); $^{230}\text{Th}/^{234}\text{U}$ dates from ref. 42 (our average of 15 results with no abnormalities, ○), ref. 43 (□), ref. 44 (△), ref. 45 (our weighted average of 2 results, ▽), and ref. 37 (○). The shaded areas indicate predictions based on known variations of geomagnetic dipole moment (Table 2 and Fig. 2).

We therefore assume that radiocarbon ages of 31,000 yr b.p. correspond to absolute ages somewhere between 36,500 and 31,000 yr BP. Strong geomagnetic fields are observed at Lake Mungo for the period 31,000–28,000 yr b.p., but the corresponding dipole moment estimates will be incorrect if the real source is not geocentric. Various eccentric source configurations are possible³¹. We assume for the present purposes that the effective dipole moment was somewhere between 1×10^{23} and 5×10^{23} A m² for a few thousand years. Radiocarbon production would be reduced from somewhere between 3.6 and 1.9 to somewhere between 1.9 and 0.7 atoms cm⁻² s⁻¹. Reservoir effects would cushion and delay the effect on ^{14}C dates. We estimate a 400–1,100 yr compression of the ^{14}C time-scale

between 31,000 and 27,000 yr b.p. (using an attenuation factor of 5–10 and a lag of $\leq 10^3$ yr, derived from ref. 36). The ^{14}C time scale would then begin to stretch in response to lower dipole moments, and radiocarbon ages at ~23,000 yr b.p. would again be between zero and 5,500 yr too young.

$^{230}\text{Th}/^{234}\text{U}$ and TL age comparisons suggest a more pronounced compression and subsequent expansion of the ^{14}C time-scale over the same period. The discrepancy is not serious but it is possible that geomagnetic effects might be reinforced by a rising and falling of sea-level around 28,500 yr b.p.³⁷.

Low values of dipole moment around 19,000–17,000 yr b.p. would cause subsequent ^{14}C ages to be between zero and 5,000 yr too young, a trend which is consistent with $^{230}\text{Th}/^{234}\text{U}$ age comparisons. Doubts about the reality of the Gothenburg excursion, together with evidence for a dipole moment comparable with the present-day one, lead us to predict a slight compression of the ^{14}C time-scale at the close of the Pleistocene. We are not yet able to say with any certainty which of the two conflicting varve chronologies (refs 40, 41; Fig. 3) is correct.

The general correspondence between the predictions and available age comparisons is such that it is not necessary to postulate long-term changes in the galactic cosmic ray flux, the solar particle flux or the interplanetary magnetic field.

Our conclusions differ slightly from those of Stuiver³⁸, mainly in that we admit the possibility of errors larger than 2,000 yr in the radiocarbon time scale. Errors of ~5,000 yr are not inconsistent with comparisons between ^{14}C and other dates, particularly when one notes the large uncertainties and the gaps in the data (Fig. 3).

The virtue in using the geomagnetic record is that changing trends (compressions or expansions) inside the overall error bounds can be predicted. Similar changes should be found for other cosmic ray-produced nuclides, and we note that the effects may be more pronounced for those species which are precipitated soon after production. Recent studies of ^{10}Be suggest that its concentration may be very sensitive to geomagnetic changes³⁹.

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Mobility and fractionation of rare earth elements during weathering of a granodiorite

H. Wayne Nesbitt

Department of Geology, La Trobe University, Bundoora, Victoria, 3083, Australia

The rare earth elements (REE) have been mobilised and fractionated during supracrustal alteration of the Torrongo granodiorite. Compared with the parent granodiorite, incipiently and moderately altered rocks are particularly enriched in the heavy REE, while the extremely altered residual products are especially depleted in the heavy REE. Mobilisation of the REE probably results from pH changes of soil and ground waters as they encounter different chemical environments while fractionation may largely result from mineralogical controls. Submarine and subaerial weathering processes may affect REE similarly, producing intensively weathered materials appreciably depleted in the REE. Spilitisation and hydrothermal alteration processes may affect basaltic rocks in much the same way as do weathering processes.

THE rare earth elements (REE) ($Z = 57-71$) have very similar chemical properties and are generally considered to be resistant to fractionation in supracrustal environments¹. This, coupled with their low solubilities, has resulted in REE distributions being used as a 'fingerprint' to help identify the parental materials of supracrustally altered rocks and sediments¹⁻³. However, mobilisation and fractionation of the REE can occur during surface alteration of rocks⁴⁻⁸. For example, kaolinite deposits derived from granite contain much lower concentrations of REE than do their parent rocks⁷ and the REE are appreciably fractionated⁷. Much evidence related to supracrustal fractionation of the REE has been obtained from studies of sediments and sedimentary rocks but the chemical compositions

of most sediments result more from physical processes (mechanical mixing) than from chemical processes^{2,9}, consequently, the response of REE to the chemical weathering process is not well documented or well understood. REE data from systematic study of a granodiorite weathering profile, ranging from fresh rock to its residue, are presented here and their significance discussed.

All REE samples were analysed using a non-destructive neutron activation technique¹⁰. Samples were irradiated for 16 h and counted 7 and 40 d after irradiation using Ge(Li) and LEPS detectors according to well established procedures¹⁰. TiO_2 , Zr and FeO_T were determined by X-ray fluorescence and sodium was measured by flame photometry. Both sodium and zirconium were checked by neutron activation techniques.

Samples of the Torrongo granite (east central Victoria, Australia) chosen for study, were obtained from a near-vertical face in a logging road cutting. Well preserved, sharp contacts separate the fresh and altered granodiorite from the clay-filled fractures (Fig. 1). The alteration intensity decreases away from the fractures often leaving unaltered 'corestones' encased in the alteration envelope (Fig. 1). The materials filling the fractures are clay minerals, a few small highly altered granodiorite fragments and minor decayed plant remains. The plant materials indicate that surface detritus, including the clays, was mechanically transported from the soil zone into the fractures. The fracture-filling clays are fine-grained and massive whereas the adjacent friable alteration zone, which also contains secondary kaolinite and illite, retains primary granitic textures, indicating *in situ* alteration. The alteration zone (Fig. 1) must have formed as a result of water percolating through a well developed soil zone, transporting suspended fine grained clays into the fractures and chemically altering the granodiorite adjacent to the fractures.

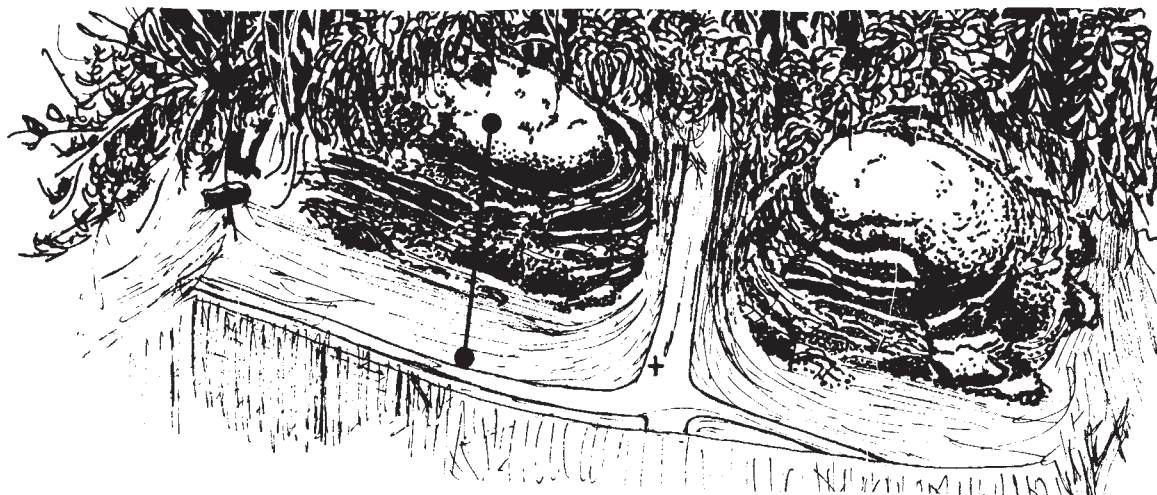


Fig. 1 The sample area: the upper part represents foliage overhanging the cutting and obscuring the soil zone. Two spherical corestones of fresh granodiorite (unshaded) are seen immediately below the foliage. These are surrounded by an exfoliation zone (heavily shaded) which is enveloped by the friable zone (thinly lined regions). Two fracture zones (essentially unshaded) intersect in the centre foreground and the near-vertical fracture separates the alteration zones about the two corestones and intersects the soil zone. Samples were taken along the vertical line terminated by two dots and the residual material sampling site is shown as a cross in the fracture zone.

The soil zone (Fig. 1) may contain allochthonous materials (wind and water transported). It is also subject to mechanical mixing due to disruption by plant roots, animal burrows and surface runoff. Hence, the fracture controlled alteration profile immediately below the solid 'corestone' was considered the best sampling area (Fig. 1).

The granodiorite is of late Devonian age, a period characterised by extensive granitic intrusion and orogenic uplift throughout southeastern Australia¹¹. The granodiorite has not had sediments or volcanics deposited on it and the area has a high annual rainfall.

REE mobilisation and fractionation during weathering

The parent material to all the samples considered is the granodiorite but as a chemical analysis yields only proportions of constituents, a method of measuring changes in concentration of elements in the alteration profile relative to those in the parent rock is needed: an immobile element could be used. If an element I is immobile, then the percentage increase or decrease of any element X in a sample (s), compared with its concentration in the parent rock (p), is given by

$$\% \text{ Change} = ((X^s/I^s)/(X^p/I^p) - 1) \times 100 \quad (1)$$

Zircon is considered a resistate mineral and accumulates as such in sediments¹². Its remarkable stability to weathering results from its exceptionally low solubility in aqueous solutions¹³. Because most zirconium in granites resides in zircon, this element should not be added to or removed from the alteration profile in significant quantities. However, because zircons may not be uniformly distributed in all samples, zirconium may introduce significant inter-sample error when calculating the percentage change of other elements. Titanium, however, is unlikely to be subject to such inter-sample variation because it is much more abundant in the rocks (present at wt % levels). Also, titanium oxy-hydroxide is the least soluble of the hydroxides and carbonates of the major and minor elements, in near neutral dilute solutions¹³; therefore, TiO₂ rather than zirconium has been chosen here as the immobile constituent (equation(1)).

Chemical analyses of zirconium and TiO₂ in the fresh rock, in materials from the alteration profile and in the fracture-filling residual clays (Table 1) are used with equation (1) to calculate the percentage changes of zirconium in the alteration profile (TiO₂ considered immobile) and the results are plotted on Fig. 2a. The data fluctuate about the 0% change value indicating that both species are effectively immobile. The fluctuations probably result from non-uniform distribution of zircons in the samples. Figure 2b shows total iron is not mobilised in the alteration profile whereas sodium is significantly depleted (Fig. 2c). However, the REE undergo striking progressive enrichments in successively more altered samples (Fig. 2d-n). The heavy REE (Z = 64-71) are more enriched (up to 300%) than the light REE (100-200% increase). Undoubtedly the REE have been added to the alteration profile and, from the nature of the enrichment trends, the fractures have acted as the avenues of transport. The source of the REE can also be identified. The residual weathering products (Table 1, sample 13), primarily kaolinite and illite, occupying the fractures, are depleted in these elements compared to the materials in the alteration profile (Table 1, Fig. 2). These relations are most obvious in Fig. 2p where samples 1 and 3 have been averaged to yield a representative parent rock (PR) and then used to normalise the data of samples 11 (alteration profile) and 13 (residual product). The fractionation patterns of, and relative abundances of the REE in samples 11 and 13 strongly suggest a close relationship between the REE concentrations of the parent rock, the materials of the alteration profile and the residual clay materials.

A simple chemical weathering scheme can be used to explain the REE enrichment in the profile and their depletion in the residue. The soil zone developed on granitoids generally contains the clay-mineral residual products of the parent

Table 1 Partial chemical analyses of selected samples* (p.p.m. except where noted)

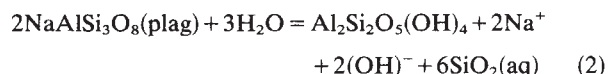
Species	(1+3)†	7	10	11	13
Zr	300.0	323.0	286.0	261.0	155.0
TiO ₂ ‡	0.885	0.86	0.81	0.78	0.41
FeO _T ‡	5.51	5.41	5.21	4.97	2.25
Na ₂ O‡	3.37	3.11	1.25	0.43	0.07
La	25.0	19.4	67.2	72.9	17.5
Ce	57.8	41.6	90.4	99.3	44.8
Nd	25.4	—	52.0	65.3	15.6
Sm	6.02	5.95	11.5	14.6	2.92
Eu	1.42	1.28	2.56	3.66	0.66
Gd	5.73	6.73	8.92	16.4	2.22
Tb	0.85	0.94	1.78	3.09	0.26
Ho	1.01	1.0	1.3	3.5	0.34
Yb	2.89	3.87	6.55	9.97	1.33
Lu	0.48	0.65	1.02	1.52	0.19

* Zr, TiO₂, FeO_T, Na₂O determined by G. Markovics (1977).

† Average value of samples 1 and 3.

‡ Analyses in wt %.

granodiorite plus decaying organic material. The pH of rain water equilibrated with atmospheric CO₂ is near 5.7. The partial pressure of CO₂ in the soil zone may be 1-2 orders of magnitude greater than atmospheric¹⁴ due primarily to decay of the organic material. Uptake of CO₂ by waters percolating through the organic-rich soil zone results in a pH decrease to values near 4 or 5. Organic acids may also be dissolved in these waters, the net result being that the soil waters are particularly aggressive¹⁴. Furthermore, the residual clay minerals, primarily kaolinite and illite, have limited capacity to neutralise the low pH waters; therefore these acidic waters percolate through the zone removing not only available soluble major and minor elements, but also some of the trivalent REE. They may be transported as carbonate or hydroxy complexes or as free ions, the dominant species being dependent on properties of the solution. Some of these acidic solutions migrate down fractures (carrying small quantities of REE) where they may contact partially altered rocks containing plagioclase, biotite and other reactive phases¹⁴. These minerals are altered to clays while at the same time the pH of the initially acidic solutions increases as shown in the following reaction:



As the solutions become more basic the relatively insoluble species such as the REE are: precipitated as hydroxides or carbonates; exchanged for H⁺ on accessible mineral exchange sites; adsorbed onto surfaces of minerals. A pH increase favours all three processes¹⁵. The scheme suggests that the parent rock is weathered to clays whereupon REE are leached and transported by acidic solutions to regions where the parent rock is being actively altered. The REE are deposited in response to pH increases during rock-water interactions at the alteration site. This simple scheme has much in common with previously proposed methods of REE depletion in residual deposits⁶.

The process explains the sources of the REE, the depletion of REE in the residual deposits and their enrichment in the alteration profile, but it does not explain their fractionation. If the above chemical alteration process is correct then the heavy REE are mobilised to a greater extent than are the light REE (Fig. 2p). The differential mobilisation may result from mineralogical stabilities and abundances as various minerals fractionate REE differently^{1,9}. The formation of some minerals, their destruction, and changes in their abundances result in the production, disappearance and changes in the number of sites that can preferentially incorporate light or heavy REE; consequently, different fractionation patterns can be produced that are entirely dependent on relative mineral abundances. Vermiculite (after biotite) and Fe-Ti oxyhydroxides (after hornblende and biotite)

are common in the exfoliation and friable zones (Fig. 1). Kaolinite and illite (after the feldspars) are also present in these zones but they dominate the residual materials where vermiculite is non-existent and the oxyhydroxides are present but greatly subordinate to kaolinite and illite. Vermiculite, Fe-Ti-oxyhydroxides, relict hornblende and biotite in the exfoliate and friable zones may contain sites that preferentially take up heavy REE while kaolinite and illite (the residual minerals) may better accommodate the light REE.

It has also been proposed that the greater mobilisation of the heavy REE (compared with the light REE) results from preferential formation of complexes with carbonate ligands⁶. This is difficult to demonstrate because the aqueous solutions are not easily sampled. Despite this, the variations in mineralogy between the parent rock, the alteration profile and the residual clay deposits suggest that the fractionation results at least in part from mineralogical controls.

Applications to sediments and sedimentary rocks

Chemical data from the Torrongo granite alteration profile indicate that fractionation does occur in the supracrustal environment. However, fractionation of the REE is not as obvious or as common in sediments and sedimentary rocks as might be expected from this study. For example, the 'average North American Shale'⁹ has been plotted on Fig. 2p (open circles) and there is no significant fractionation of different REE relative to those in the fresh granodiorite. (The Torrongo granite has a chondrite normalised pattern that mimics BCR-1.) The lack of fractionation in sediments is explained by the fact that most sediments result from mechanical mixing of detritus and hence tend to be homogenised. If sample 11 (Fig. 2p), representative of the partially altered granodiorite, and sample 13 (Fig. 2p), representative of the residual materials, were mechanically

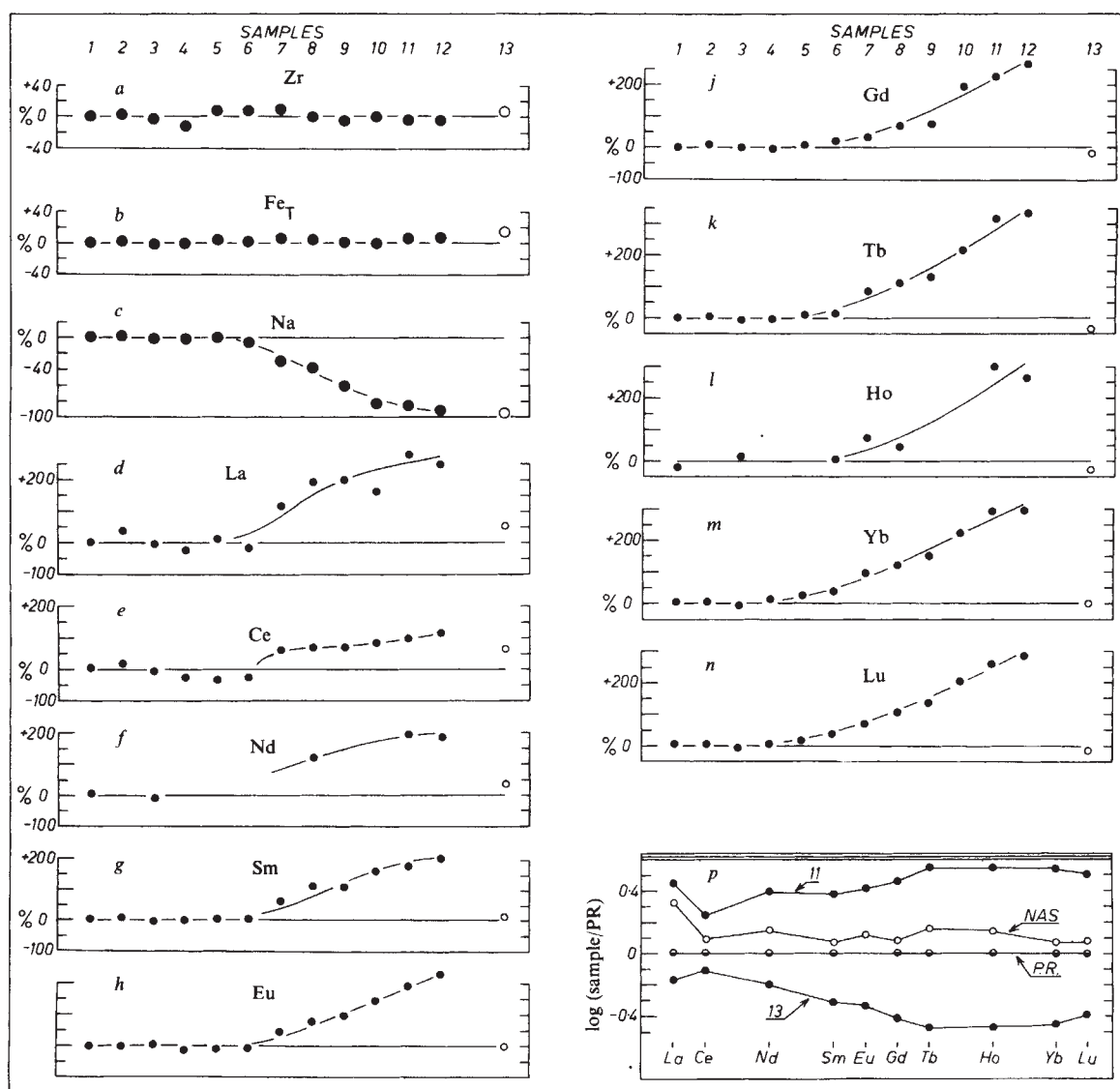


Fig. 2 The percentage change of elements is shown relative to the 'immobile' species TiO_2 . The ordinate is given by equation (1) and the abscissa shows the individual samples ordered according to the degree of chemical alteration. The alteration index (AI) used is $\text{Al}_2\text{O}_3\text{--Na}_2\text{O--K}_2\text{O--CaO}$. The greater the AI value, the more altered is the sample. The AI reflects essentially the alteration of the feldspars in the rock. The spatial sequence from corestone to the edge of the fracture was 2, 1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 11 which is similar to the above AI order. The freshest rock plots at the extreme left side. ●, Samples from the alteration profile; ○, the fracture-filling residual material. p, the REE patterns of the fresh parent rock (PR samples (1 + 3), ●) the altered granodiorite (sample 11, ●) the residual materials (sample 13, ●) and the average North American shale (NAS, ○).

mixed in approximately equal proportions, the resulting REE pattern would be similar to the fresh granodiorite and the 'average North American Shale'. Apparently, REE patterns in sediments may be indicative of parent material only when all fractions of the weathering products derived from the parent rock are included in the sediment sample.

The fractionated REE patterns observed in sediments² can be explained in two ways. The residual materials in the soils and fractures of the Torrongo granite are very fine-grained clays whereas the friable alteration zone contains appreciable medium- and coarse-grained quartz, feldspar and rock fragments. Size sorting of this outcrop, in a sedimentary environment, would probably produce a sandstone with a light REE depleted pattern and a kaolinite-illite-rich shale enriched in the light REE. Alternatively, the denudation of a cratonic mass containing a well developed weathering profile would first remove and deposit the residual clay materials. Only after removal of the residual soils would the partially altered materials be exposed and removed in quantity to be deposited elsewhere. The two sediments could well display fractionated REE patterns.

Other studies

There has been no other similar subaerial weathering study although the report of Burkov and Podporina⁷, in which they obtained REE patterns of residual granitic materials, is closely allied to the present one. In their report the sampling control and normalisation techniques, which here are important for the interpretation of the data, were not applied making direct comparisons qualitative. They observed that the REE concentrations of the kaolin residues were lower than in the parent granitoids; in this respect the two investigations agree. In contrast to the present study they noted that the light REE/heavy REE values were lower in the kaolinite residues than in the present granites. The weathering residues and the parent rocks in the two studies may be substantially different with respect to their mineralogies or with respect to their mineral proportions. The discrepancy in REE distribution patterns may result from these differences but additional studies are needed to verify the suggestions.

Rare earth element behaviour during seawater alteration of basaltic materials (in both hydrothermal, and submarine weathering conditions) has been the subject of several studies. Frey *et al.*¹⁶ analysed both fresh basaltic glass and its palagonised equivalent. The palagonite REE pattern normalised to the parent glass (Fig. 3a) is similar to that of the granodiorite residue (Fig. 2p, specimen 13). As with the residual granitic materials, the palagonite is depleted in the REE and particularly in the heavy REE. Frey *et al.*¹⁶ observed similar patterns when comparing altered crystalline rock to fresh glass. Ludden and Thompson¹⁷ have also studied the behaviour of REE during low temperature seawater alteration of pillow basalts. The parent basalt compositions of the altered materials is not precisely known in their study, but their compositions of a fractionated and unfractionated basalt should encompass the compositions of the parent rocks to the altered materials. The unfractionated and fractionated basalts have been used to normalise the patterns of the altered materials (Fig. 3b and 3c). Relative to both the fractionated and unfractionated basalt the altered interiors are relatively enriched in the light REE compared with the heavy REE. The outer altered rims of the pillows have not been treated here as they may be contaminated by manganese crusts (Ludden and Thompson unpublished data). There is undoubtedly appreciable fractionation of the lanthanides during both subaerial and submarine weathering (Fig. 2p, 3a, 3b, 3c). The pattern for the granodiorite residue (Fig. 2p, specimen 13) results from leaching of all lanthanides with preferential removal of the heavy elements: the pattern of Fig. 2p (specimen 13) should, therefore, be referred to as a heavy REE depleted pattern. The patterns in Fig. 3a, b and c may also result from preferential removal of heavy REE from the altered materials. Frey *et al.*¹⁶ supply zirconium values for the palagonite and the

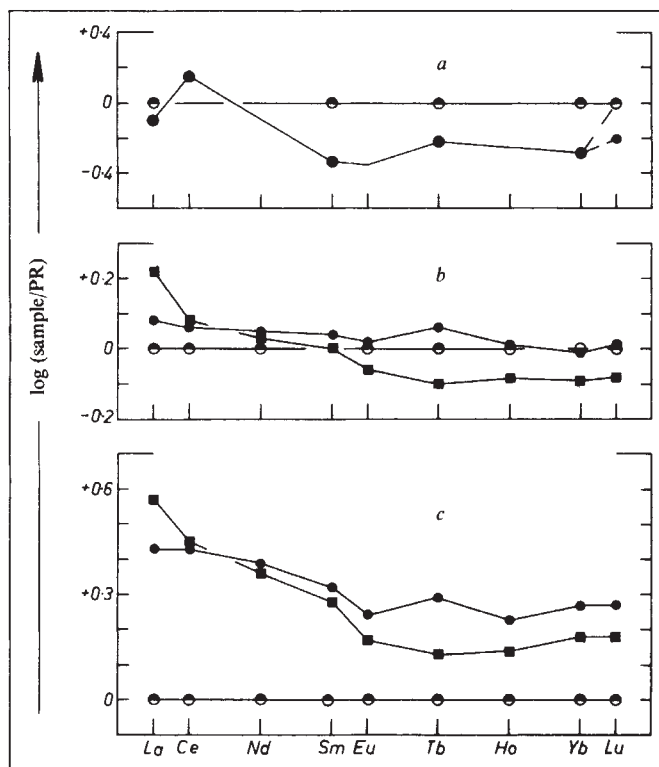


Fig. 3 a, The palagonite pattern (●) normalised to the parental fresh glass (○). The data are taken from Frey *et al.*¹⁶. b, Altered interior of 2.5 Myr old (●) and 5.0 Myr old (■) pillow interiors normalised on a fresh fractionated basalt (○) from the same area. The data is from Ludden and Thompson¹⁷. c, as b except the altered pillow interiors are normalised on fresh unfractionated basalt from the area of the pillows where again the data is taken from Ludden and Thompson¹⁷.

fresh parent glass and they contain 95 and 90 p.p.m. Zr respectively. If zirconium is taken as immobile then all lanthanides, with the exception of Cerium, have been removed from the glass during palagonisation and the heavy REE have been most strongly leached. As with the granodiorite residue pattern the palagonite pattern should be referred to as heavy REE depleted. Unfortunately the same calculations cannot be made on the data of Ludden and Thompson because of the ambiguity concerning the compositions of the parent materials.

Wood *et al.*¹⁸, Hellman and Henderson¹⁹ and Humphris *et al.*²⁰ suggest that spilitised and hydrothermally altered basalts display REE fractionated patterns when compared with the source basalts. In each study the patterns are, enriched in the light REE relative to the heavy lanthanides. In this respect the patterns are similar to the residual products of the granodiorite and the palagonite¹⁷. However, the light REE-enriched spilites and hydrothermally altered basalts¹⁸⁻²⁰ may result from appreciable addition of the light REE to the altered rocks, preferential removal of the heavy REE or a combination of both processes. Additional studies of these rocks may be necessary to determine which of the processes are dominant.

These studies¹⁸⁻²⁰ suggest that the primary and/or secondary mineralogy greatly influence the fractionation of the lanthanides during alteration of the rocks. These suggestions are again compatible with the observations made here concerning weathering of the Torrongo granite. Comparing this study with the others¹⁶⁻²⁰ suggests that there may be similarities between intensive weathering processes (subaerial and submarine) and spilitisation and hydrothermal alteration processes.

Cullers *et al.*²¹ studied REE distributions in the clay-sized fraction of shales. They suggested that the differences in REE contents between samples of different localities may result from

chemical weathering processes. The suggestion is strongly supported by this study. The extent of chemical weathering and subsequent transport history of the sediment derived from even the same source materials may show appreciable variations in REE concentrations and in REE fractionation patterns. Cullers *et al.*²¹ postulate that REE contents of clay minerals are determined primarily by the REE content of the source rock. However, at least in the granodiorite alteration profile, the weathering products contain REE concentrations appreciably different from the source rock. Cullers *et al.*²¹ also noted an europium anomaly for the clays when normalised to NAS: no anomaly results from the weathering of the granodiorite. If the findings of this study are representative of subaerial weathering then the observed europium anomaly results from an europium anomaly of the source material or possibly from diagenesis through reduction of Eu^{3+} to Eu^{2+} (by carbonaceous material) and subsequent leaching of europium from the clays. In fact the wide variation of REE distributions found in the clay mineral groups studied by Cullers *et al.*²¹ may, in large part, result from post-depositional processes.

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Conclusions

I have shown that the REE can be mobilised in the supracrustal environment; that the enrichment and depletion of the REE are probably related to the pH of ground waters; that the fractionation of REE is probably a result of primary and secondary mineral abundances. The REE patterns of sediments and sedimentary rocks generally do not show the strong REE fractionation patterns observed in the altered materials studied here. The mechanical mixing of sedimentary detritus probably homogenises weathered materials to the extent that extreme fractionation patterns are not normally observed. Comparisons between this study and other studies suggest that subaerial and submarine weathering processes, and perhaps even hydrothermal processes, affect lanthanides similarly.

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Growth rate influence on the chemical composition of phytoplankton in oceanic waters

Joel C. Goldman*, James J. McCarthy† & Dwight G. Peavey*

* Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

† Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

The chemical composition of oceanic phytoplankton (by atoms) typically occurs in the proportions $\text{C}_{106}\text{N}_{16}\text{P}_1$. Yet, in laboratory growth conditions these proportions are only observed for marine phytoplankton at high growth rates when non-nutrient limitation is approached. Thus growth rates of natural phytoplankton populations in oceanic waters may be near maximal and hence non-nutrient limited. The uniformly low biomass and residual nutrient levels in such waters does not preclude the possibility of high growth rates because zooplankton grazing and nutrient regeneration within the euphotic zone may keep this highly dynamic system in a balanced state.

THERE is a large temporal and spatial variability of nutrient cycling in the surface waters of the oceanic environment¹. Concomitant with the highly dynamic turnover of nutrients in oceanic surface waters are four characteristics of marine nutrient chemistry^{2–5}. These are: (1) both phytoplankton biomass and aqueous nutrient concentrations are uniformly low in areas where there is little seasonal variation in the depth of the mixed layer; (2) the composition of particulate matter in marine waters is usually in the approximate proportions 106:16:1 (by atoms) for the three major elemental constituents, carbon,

nitrogen and phosphorus (commonly referred to as the 'Redfield ratio'); (3) the dissolved inorganic N:P ratio of oceanic waters below the thermocline and in many coastal waters preceding bloom conditions is often about 16:1; (4) during bloom conditions inorganic nitrogen and phosphorus may disappear from solution in approximately a 16:1 ratio.

There are, however, numerous exceptions to these generalisations^{3,4}. For example, within the euphotic zone phosphorus can be recycled more rapidly than nitrogen^{6,7}, so that a small residual of inorganic phosphorus is sometimes observed when inorganic nitrogen is undetectable⁸. Similarly, differential rates of nitrogen and phosphorus cycling preclude any *a priori* inferences as to the chemical composition of phytoplankton from the rates of disappearance of nutrients from surface waters⁹. Finally, particulate matter in surface waters contains varying amounts of non-phytoplankton material, ranging from a large percentage in nutrient-poor oceanic waters to a considerably smaller amount in productive coastal and upwelling systems¹⁰.

There is, nevertheless, an abundance of data from recent literature on the chemical composition of phytoplankton in both productive and unproductive waters^{6,11–17} to support the earlier conclusions of Redfield² and Fleming¹⁸ that there is a striking consistency in the chemical composition of marine phytoplankton. For this discussion, however, considerable flexibility is allowed in the proportions of the chemical constituents without

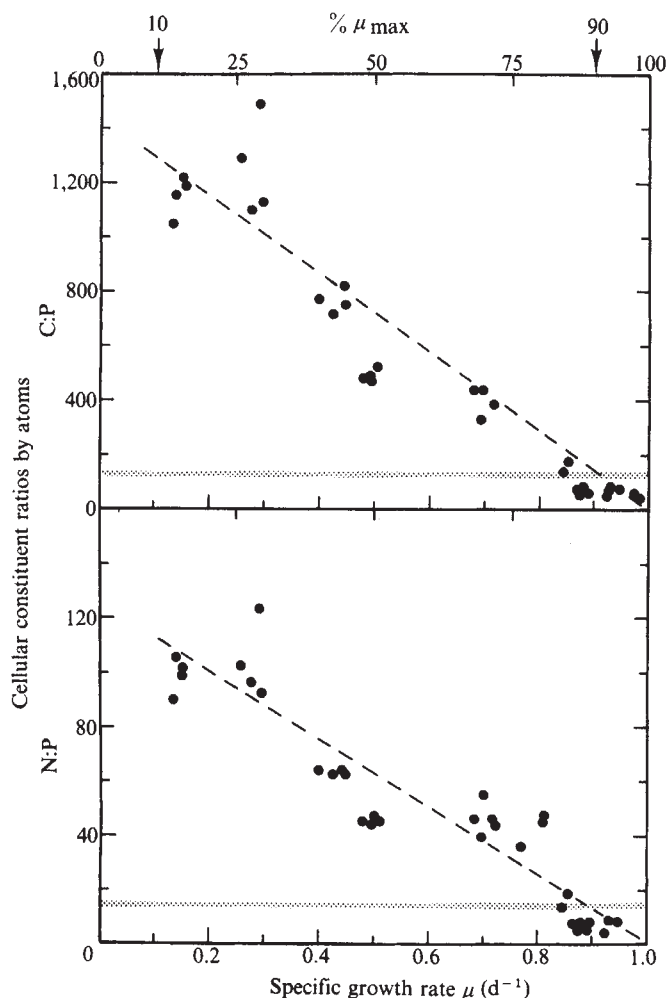


Fig. 1 The effect of specific growth rate on the carbon:phosphorus and nitrogen:phosphorus ratios of *Monochrysis lutheri* under phosphorus limitation in continuous culture at 18 °C and 0.03 cal cm⁻² min⁻¹ light intensity. Medium N:P ratios = 87:1 to 412:1. Shaded lines in Figs 1–3 represent C:P and N:P components of Redfield ratio (C₁₀₆N₁₆P). All dashed lines in these figures were drawn by eye and represent trends not absolute relationships between the respective cellular chemical ratios and growth rates. $\mu_{\max} = 0.95 \text{ d}^{-1}$.

detracting from the generalisation of a uniform phytoplankton chemical composition. For example, values of ~75:1 to 150:1 for the C:P ratio and ~10:1 to 20:1 for the N:P ratio are consistent with the concept of a uniform chemical composition.

A clear distinction must be made between the processes controlling nutrient and phytoplankton interactions in productive coastal and upwelling waters and those in oceanic environments. We consider here only the latter system. By comparing the results of laboratory experiment on cultured marine phytoplankton with field observations, we propose a relationship between the nutrient-influenced growth rate and the elemental composition of oceanic phytoplankton.

Nutrient fluxes in the oceanic environment

On a long-term basis the dampening effect of the large ocean reservoir leads to a uniformity in the cycling of nitrogen and phosphorus. To maintain this consistency the major sources of nitrogen and phosphorus for phytoplankton growth in the euphotic zone of oceanic environments must be in balance with the major sinks of these nutrients on a long-term basis. Thus, fluxes of nutrients from vertical upward transport of nutrient-rich water across the thermocline, bacterial degradation of

animal faeces and other detritus within the euphotic zone, and excretion of soluble nutrients by zooplankton and fish must be balanced by the assimilation of these nutrients by phytoplankton plus the loss of organisms and detritus to deep waters. Other nitrogen flux terms such as rain input, N₂ fixation and river discharges, and loss through permanent sediment burial and denitrification with evolution of N₂O and/or N₂, are essential components of a global nitrogen budget; but, at steady state these fluxes must cancel. In this regard, both rain and N₂ fixation provide an extremely small fraction of the nitrogen requirement for phytoplankton in the north central Atlantic¹⁹, whereas river and waste water discharges are important sources of nitrogen and phosphorus in coastal waters⁸. But, because of the extent of the oceans, the overall impact of these latter additions on the global marine nutrient budget, and, in particular, on nutrient inputs to the oceanic mixed layer, has been negligible.

Mechanisms controlling marine nutrient chemistry

The generality of the Redfield ratio for phytoplankton and the 16:1 ratio for dissolved inorganic nitrogen and phosphorus in deep water leads to the apparent conclusion that nitrogen and phosphorus simultaneously influence phytoplankton growth in the oceans. But does the chemistry of the oceanic environment control the physiology and resulting chemical composition of the phytoplankton, or is the nutrient chemistry of the oceans manipulated by rather rigid physiological requirements?⁴ The answer to this is not simple. A major complication is the often demonstrated fact, beginning with Ketchum's classical experiments on P-limited growth of *Nitzschia closterium*²⁰, that in laboratory conditions the chemical composition of phytoplankton can vary appreciably as a function of nutritional state and other growth conditions^{16,21,22}.

Growth rate effects on phytoplankton chemical composition

To establish the environmental conditions in which the chemical composition of marine phytoplankton can be characterised by the Redfield ratio, we grew three marine phytoplankton species in continuous culture: the chrysophyte *Monochrysis lutheri* under P-limitation and with NO₃⁻-N as the nitrogen source, the diatom *Thalassiosira pseudonana* 3H under NH₄⁺-N limitation, and the chlorophyte *Dunaliella tertiolecta* under NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, urea-N, and P-limitation. Details of the experimental procedures are reported elsewhere^{23–25}. Although the

Table 1 Cellular constituent ratios of marine phytoplankton grown on varying nitrogen:phosphorus ratios and at different growth rates

Nutrient limitation	Species (clone)	Culture N:P ratio (atoms)	% $\mu_{\max}$	Cellular ratios (atoms)	
				C:N:P	C:N
Phosphorus	<i>Monochrysis lutheri</i> (Mono)	87–412	10	1,300:115:1	11.3
			50	720:65:1	11.1
			90	106:15:1	7.1
	<i>Dunaliella tertiolecta</i> (Dun)	50	10	600:48:1	12.5
			50	325:32:1	10.2
Nitrogen	<i>Thalassiosira pseudonana</i> (3H)	5	10	63:5:1	12.6
			50	68:7:1	9.7
			90	106:15:1	7.1
	<i>Dunaliella tertiolecta</i> (Dun)	5	10	85:5:1	17.0
			50	60:5:1	12.0
			90	35:5:1	7.0
		10	10	160:10:1*	16.0
			50	120:10:1	12.0
			90	70:10:1*	7.0
			15	300:15:1	20.0
			50	175:15:1	11.7
			90	106:15:1*	7.1

* Extrapolated from curves in Fig. 2.

concentrations of nutrients in the media delivered to the growth chambers were high, the residual limiting nutrients in the cultures over most of the growth rate range remained at levels similar to those typical of oceanic mixed layers.

The effect of growth rate on the cellular C:P and N:P ratios was a distinct function of the degree and type of nutrient limitation. For P-limited growth of both *M. lutheri* and *D. tertiolecta* there was a large decrease in the cellular nutrient ratios from 600:1 to 1,000:1 for C:P and 50:1 to 100:1 for N:P at one end of the growth rate spectrum ($\sim 10\%$ $\mu_{\max}$) to respectively 106:1 and 15:1 at the other end ($\sim 90\%$ $\mu_{\max}$) (Figs 1, 2). In contrast, the cellular ratios varied quite differently for the N-limited cultures. For *T. pseudonana*, in which a single medium N:P ratio of 5:1 was used, the cellular ratios increased from 65:1 for C:P and 5:1 for N:P below 10% $\mu_{\max}$ to respectively 120:1 and 15:1 above 90% $\mu_{\max}$ (Fig. 3). In the case of N-limited growth of *D. tertiolecta*, there was no effect of either nitrogen source or growth rate on cellular N:P ratios, indicating complete assimilation of both nutrients (Fig. 2). On the other hand, the cellular C:P ratios all decreased with increasing growth rates. At 10% $\mu_{\max}$ the C:P ratios were 300:1, 160:1, and 85:1 for the respective medium N:P ratios of 15:1, 10:1, and 5:1. The Redfield C:P ratio of 106:1 was

approached at 90% and 60% of $\mu_{\max}$ when the medium N:P ratios were respectively 15:1 and 10:1. In contrast, for a medium N:P ratio of 5:1 the cellular C:P ratio was always below the Redfield proportion and decreased to 35:1 at 90% $\mu_{\max}$.

Factors influencing the Redfield ratio

A common feature to the three growth experiments was that the Redfield ratio was approached only at high growth rates (Table 1). With the exception of the one *D. tertiolecta* experiment, this trend was true regardless of the medium N:P ratio. Based on the results of our previous studies²³⁻²⁵, at 90% $\mu_{\max}$ discernible nutrient limitation did not exist in any of the cultures. Hence, attainment of the Redfield ratio seem to coincide with conditions of near to complete nutrient saturation. In addition, the C:P and N:P ratios were considerably more affected by growth rate under P-limitation than when N was limiting, for example, 5–10-fold changes for P-limitation and only 2–3-fold variations for N-limitation (Table 1). As exemplified by the *M. lutheri* results, variations in the medium N:P ratio (87:1 to 412:1) did not affect the magnitude of the cellular N:P ratios when phosphorus was limiting. Yet, when nitrogen was limiting the magnitude of the medium N:P strongly influenced the resulting

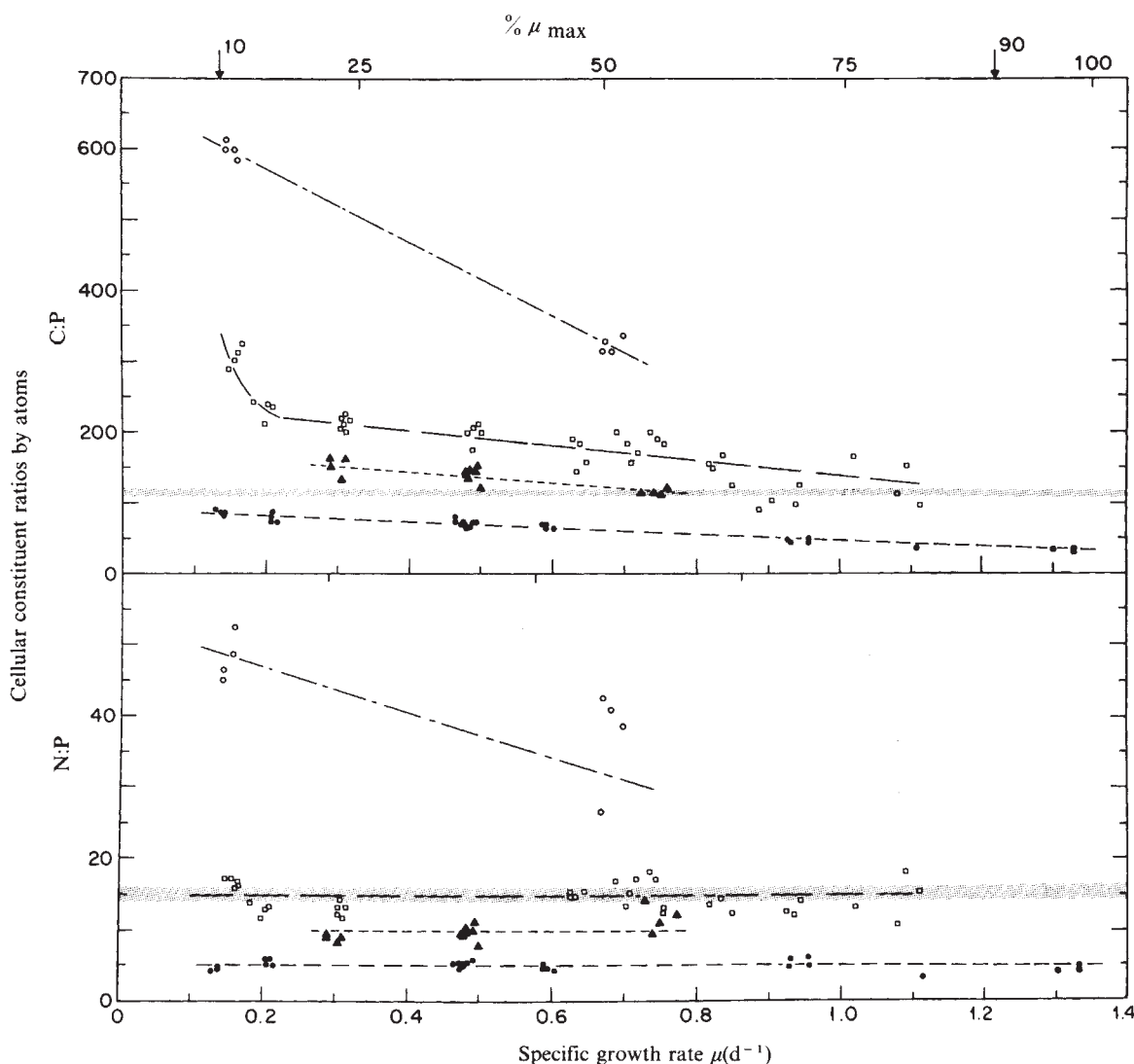


Fig. 2 The effect of specific growth rate on the C:P and N:P ratios of *Dunaliella tertiolecta* under phosphorus and nitrogen limitation in continuous culture at 19°C and $0.06\text{ cal cm}^{-2}\text{ min}^{-1}$ light intensity. Phosphorus limitation is presumed for medium N:P $\geq 50:1$ and nitrogen limitation for N:P $\leq 15:1$. ○, medium N:P = 50; □, medium N:P = 15; ▲, medium N:P = 10; ●, medium N:P = 5:1. The source of nitrogen had no effect on the relationships between cellular ratios and growth rates, as each cluster of four data points at a particular growth rate represents the four nitrogen sources used: NH_4^+-N , NO_2^--N , NO_3^--N , urea. $\mu_{\max} = 1.35\text{ d}^{-1}$.

cellular N:P ratios. For *T. pseudonana* at low growth rates and for *D. tertiolecta* at any growth rate the medium and cellular N:P ratios were identical. In contrast, the C:P ratio typically approached the Redfield value only in the high growth rate regions. Similar results have been attained by other workers^{16,21,26,27}.

Biological effects

A possible explanation, therefore, as to why the chemical composition of natural marine phytoplankton is typically characterised by the Redfield ratio, is that growth rates in marine waters may be quite high. The corollary to this argument is that if phytoplankton growth rates are high, then nutrient availability is not the prime limiting factor in these waters.

Before examining the above hypothesis, it is instructive to consider first the available growth rate data for natural marine waters. Such measurements are difficult, and, as summarised in Table 2, the data are amazingly scant and conflicting; thus it is virtually impossible to draw any concrete conclusions as to the magnitude of *in situ* growth rates of oceanic phytoplankton. For example, Eppley⁴⁶ concluded that there was a direct correlation between the degree of eutrophy in marine waters and growth rates. Other workers^{31,44,45}, however, have found an opposite trend: higher growth rates associated with nutrient-poor and unproductive waters (Table 2). These contradictory conclusions are not amenable to close scrutiny because different procedures were used, and there is no completely satisfactory technique for making accurate estimates of *in situ* growth rates.

Hence, growth rates of marine phytoplankton are typically either <0.5 doublings per day ($\mu = 0.35 \text{ d}^{-1}$) or >1 doubling per day ($\mu = 0.69 \text{ d}^{-1}$)^{32,34,44-46}. However, it is % $\mu_{\max}$, rather than the absolute value of μ that is germane to the question of the Redfield ratio. This point is exemplified by our observation that, although $\mu_{\max}$ for the three species in the experiments described was distinctly different, the Redfield ratio was attained at growth rates close to $\mu_{\max}$ in each case. The magnitude of $\mu_{\max}$, aside from being species specific, is also a function of temperature and light^{46,47}. Therefore, growth rate data for natural populations, such as compiled in Table 2, provide no insight as to how fast these populations were growing relative to their potential maximum rates at the time of sampling.

The idea then that phytoplankton growth rates in oceanic waters are generally high and close to $\mu_{\max}$ is at first difficult to accept, particularly when there is overwhelming evidence that nutrients in these waters are in such short supply and are often undetectable. To examine whether high growth rates and low nutrient levels can occur simultaneously, it is useful to consider the simple continuous culture as a dynamic system analogous to a segment of the open ocean. In such a system a limiting nutrient plus other nutrients in excess are added to the culture vessel at a fixed dilution rate (culture displacement per unit time). Cells assimilate the limiting nutrient and grow to a specific population size. Eventually a steady state is achieved in which the biomass produced in the culture per unit time is balanced by the discharge of biomass. An important result, often misunderstood, is that the growth rate of the organisms is then equal to the dilution rate, but it remains completely independent of the concentration of limiting nutrient in the medium feed. However, the steady state biomass is proportional to the concentration of added limiting nutrient²³.

In steady state conditions there seems to be a common trend of virtually undetectable residual limiting nutrient levels over the entire growth rate spectrum until just before the maximum growth rate is reached, regardless of which nutrient is limiting^{23,24,48}. This common feature of marine phytoplankton physiology suggests that these organisms have a very high affinity for nutrients; thus at steady state it is possible to have simultaneously low or undetectable residual nutrient levels and high growth rates regardless of the biomass concentration.

On a long-term basis the euphotic zone of the open ocean probably approximates a continuous culture at steady state as well or better than any other aquatic system³²; nutrient levels

are uniformly low and the phytoplankton biomass is relatively invariant with time. Because nutrient input by vertical transport across the thermocline is generally considered to be small in oceanic systems (1–10% of the phytoplankton demand)^{49,50}, the main and preferred sources of nutrients (such as, NH_4^+ , urea) are derived from animal excretion and bacterial mineralisation of detritus and dissolved organic matter^{32,51,52}. At the same time phytoplankton biomass is continually cropped by zooplankton grazing. Hence, the zooplankton and bacteria act as the input and overflow mechanisms of the continuous culture³². The important question, however, for which there is no clear-cut answer is: at what rate does this dynamic system function?

The possibility that pelagic planktonic production rates could be near $\mu_{\max}$ is compatible with observations of both low biomass and low residual nutrient levels, and with the notion that a particular nutrient limits the total biomass of the biological system rather than the actual rate processes. Cushing⁵³, in fact, argues that the euphotic zone of the open ocean is a relatively closed system, functioning at a very high rate with small temporal and spatial amplitudes in standing crop and nutrient levels; and it is the combination of zooplankton grazing and nutrient regeneration within the euphotic zone that is responsible for this tight coupling between trophic levels. Vertical transport of nutrients across the thermocline, although thought to be a minor source of nitrogen for phytoplankton^{49,50}, can at times be significant due to internal mixing⁵⁴. Such pulsed inputs of nutrients to the mixed layer probably lead to proportional increases in standing crop, but, as shown above, do not necessarily affect the gross rate processes.

The microscale of nutrient cycling

Although the above arguments are appealing for describing a steady state oceanic system, we know from recent studies⁵⁵ that phytoplankton growth rates and transient nutrient uptake rates may be uncoupled. For example, we showed that *T. pseudonana* (3H) grown in continuous culture under modest NH_4^+ -N limitation, can assimilate this nutrient at a rate up to 30 times its

Table 2 Summary of available phytoplankton growth rate data in doublings per day for natural marine waters

Location	Growth rate doublings per day*	Ref.
Sargasso Sea	0.26†	28
Florida Strait	0.45†	28
Carolina Coast	0.37†	28
Montauk Pt, L.I.	0.35†	28
S Calif. Coast	0.25–0.4†	29
S Calif. Coast	0.7†	30
NW Atlantic	0.2–1.7	31
N Pacific	0.2–0.4	32
Northern N Pacific	0.36–0.89	33
North Sea	0.67–1.33	34
Sargasso Sea	0.05–0.14	35
Tyrrhenian Sea	0.07–0.25	35
Baja, Calif. Coast	0.2–1.4	36
Peru Current	0.67†	37
Peru Current	0.73†	38
SW Africa Coast	1.0†	39
W Arabian Sea	>1.0 †	40
Narragansett Bay, RI	0.4–1.94	41
Narragansett Bay, RI	<0.1 –3.8	42
Santa Monica Bay, Calif.	0.3–0.7	43
Sargasso Sea	8	44
Oligotrophic Waters	6.6	45
Mesotrophic Waters	2.3	45
Eutrophic Waters	0.14	45

No distinction is made between coastal, upwelling and oceanic waters. The lack of correlation between growth rate and degree of productivity attests to the confusion regarding growth rate measurements and their meaning.

* Doublings per day $\times 0.693$ = specific growth rate μ .

† Obtained from Table 2 in ref. 46.

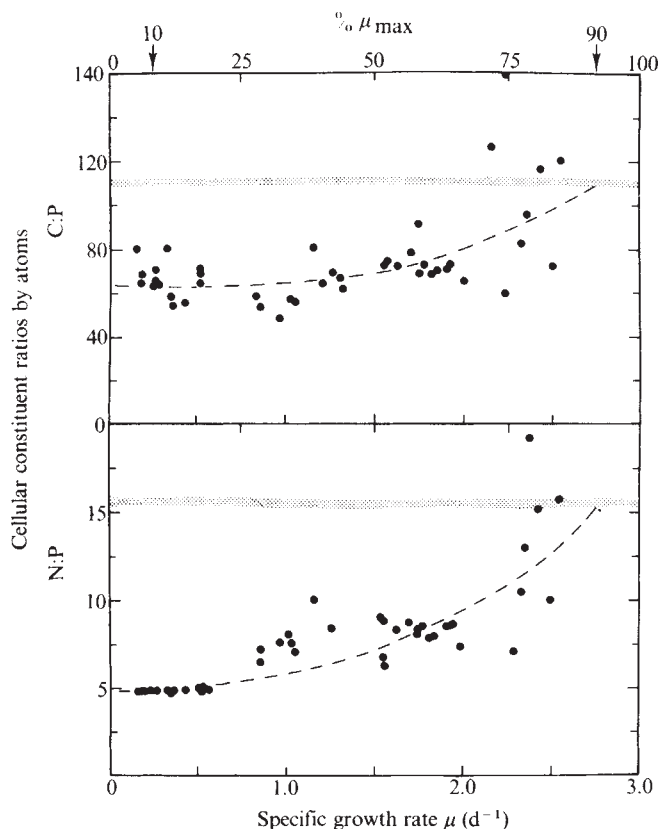


Fig. 3 The effect of specific growth rate on the C:P and N:P ratios of *Thalassiosira pseudonana* 3H under ammonium limitation in continuous culture at 19°C and 0.06 cal cm⁻² min light intensity. Medium N:P ratio = 5:1. $\mu_{\max} = 3.02 \text{ d}^{-1}$.

specific growth rate when exposed to saturating concentrations of NH_4^+ for short (5 min) periods⁵⁵. The major implication of this finding is that a nutrient-stressed phytoplankton cell need be exposed to an elevated nitrogen concentration for only a small fraction of its doubling to obtain a significant ration of this nutrient. Similar data for phosphorus uptake are available, indicating that the dynamics of phosphorus assimilation are similar to those for nitrogen⁵⁶.

This situation could exist on a microscale in which a phytoplankton cell randomly and perhaps frequently passes within a zone of elevated NH_4^+ , urea, or PO_4^{3-} concentration surrounding a zooplankton or bacterial assemblage. This hypothesis is consistent with the observation that nutrient concentrations in oceanic surface waters are frequently below detectable levels when, on the basis of measured photosynthetic rates, there is no indication of serious nutrient limitation⁵⁵. From this perspective, the response of an individual oceanic phytoplankton is quite unlike that of the total population maintained at steady state in a continuous culture. By enhanced nutrient uptake the nutrient-stressed cell can rapidly attain the nutrient ration necessary to synthesise cellular material at maximal rates. A more complete understanding of the nutrition of these organisms in nature will

require laboratory culture procedures which mimic the short-term response of the individual cell to a transient nutrient regime.

Although these processes actually occur on a temporal and spatial scale that we cannot observe, the net sum of all these events appears as a 'steady state' ocean system, but on a much larger scale. Thus the hypothesis that the ocean system is in a highly dynamic state, characterised by high rates of phytoplankton growth and nutrient turnover, is entirely consistent with our experimental results showing attainment of the Redfield ratio when phytoplankton growth rates and growth rate potential are close to $\mu_{\max}$ at essentially undetectable nutrient concentrations. Unfortunately, the major limitation of this hypothesis is that the supporting evidence is highly circumstantial. Only when accurate measurements of *in situ* growth rates are possible will the question be answered adequately.

Conclusions

The possibility that the N:P ratio of 16:1 in the bulk of the world's oceans is the result of a combination of geochemical processes controlling the input of phosphorus and microbial processes such as N_2 fixation, nitrification, and denitrification controlling nitrogen inputs cannot be discounted. That phytoplankton can simultaneously strip nitrogen and phosphorus from solution when the aqueous N:P ratio varies from 5:1 to 15:1, is consistent with the hypothesis that the chemical composition of marine phytoplankton is strongly influenced by the chemistry of the surrounding waters, irrespective of growth rate. Yet, the concept of geochemical control of the chemical composition of phytoplankton does not explain why inorganic carbon, which is present in quantities far in excess of the requirements of marine phytoplankton, appears to be assimilated in the Redfield proportions only at high growth rates (Table 1). This effect can be seen most clearly by observing the C:N ratios under N-limitation (Table 1). Whereas the N:P ratios are relatively invariant over the growth rate spectra (Figs 1–3), the C:N ratios, particularly those for *D. tertiolecta*, vary significantly. Clearly the carbon component of the Redfield ratio is controlled primarily by physiological and not aqueous chemical factors.

Our results demonstrate the circumstances in which the Redfield ratio can be attained, and point to the possibility that the rate processes in nutrient impoverished oceanic regions may be in a highly dynamic and balanced state. One conclusion that can be stated with some certainty is that severe phosphorus limitation probably does not exist in the world's oceans. Otherwise the C:P ratios of phytoplankton would be far larger than the typically observed values of 75:1 to 150:1. However, to explore further these questions involves new approaches for studying microbial interactions on temporal and spatial scales that are far smaller than were previously assumed to be important.

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A mutation altering the function of a carbohydrate binding protein blocks cell-cell cohesion in developing *Dictyostelium discoideum*

Jasodhara Ray*, Thomas Shinnick & Richard Lerner

Scripps Clinic and Research Foundation, Department of Cellular and Developmental Immunology, 10666 N. Torrey Pines Road, La Jolla, California

In *Dictyostelium discoideum*, carbohydrate binding proteins (CBPs) or lectins have been implicated in the molecular basis of cellular cohesion. To determine the role of these CBPs, we have attempted to isolate structural gene mutants in which the CBPs have a defective affinity for carbohydrate ligands. We now report the isolation of a spontaneous, cross-reacting material (CRM) mutant which is non-cohesive and fails to develop. The mutant seems to have a defect in the structural gene for one of the two developmentally regulated carbohydrate binding proteins (CBP-26), which renders it unable to bind to galactose-containing ligands. The fact that wild-type cells interact with the mutant and carry it through development strongly supports a model of cell-cell interaction in which cohesion is mediated by complementary molecules.

AN important goal of biology is an understanding of the molecular basis of the cell-cell interactions which underlie morphogenesis and development. In several developmental systems, specific molecules have been proposed as possible mediators of cell-cell adhesion¹⁻⁸. The candidate molecules are usually said to mediate specific cellular adhesions because either the purified molecule causes cell aggregation^{3,9-17}, or antibodies against the molecules block cellular adhesions or even development¹⁸⁻²⁴. However, although intriguing, these studies are indirect. Similarly, in the cellular slime mould *Dictyostelium discoideum*, our own studies and those of Rosen *et al.*²⁵ have defined two developmentally regulated carbohydrate binding proteins (CBPs) which have been suggested to be involved in specific cell-cell adhesion. The CBPs are developmentally regulated and appear on the cell surface when the cells become cohesive^{25,26}. In their native form they are homotetramers, with molecular

weights of about 100,000. On reduction and separation of their subunits in SDS-acrylamide gels, the two CBPs can be distinguished from each other by the molecular size of their subunits [26,000 (CBP-26) and 24,000 (CBP-24) (ref. 27)]. Structural and kinetic studies have shown that CBP-26 and CBP-24 are distinct gene products with a very similar time course of appearance during development²⁷⁻²⁹. However, as for the candidate molecules of other systems, the evidence that the CBPs of *D. discoideum* have a role in development is largely indirect³⁰.

We now report the isolation of a spontaneous cross-reacting material (CRM) mutant which seems to have a defect in the structural gene for CBP-26. The mutant is non-cohesive, suggesting a critical role for CBP-26 in cellular cohesion and subsequent development. The finding that when mutant cells are mixed with wild-type cells, the mutant develops normally (synergy), is strong evidence for a model ('lectin-ligand') in which complementary molecules mediate specific cell-cell cohesion.

Isolation of HJR-1

A population of NC-4 cells was enriched for cells lacking CBPs by affinity chromatography on Sepharose 6MB coupled to desialated fetuin. Whereas wild-type cells bind to the galactose matrix through their CBPs, cells lacking CBPs do not bind (J.R., unpublished). Wild-type NC-4 cells were collected after 16 h of development (when the amount of cell surface CBPs is maximal), and 10⁸ cells were chromatographed on a 4-ml Sepharose 6MB column. About 0.3% of the cells came out during column washing. Two hundred of the non-binding cells were plated out in association with *Klebsiella aerogenes* and allowed to grow and develop at 22 °C. One variant plaque that failed to develop normally was picked for further study and purified by successive single-plaque isolations; the mutant strain was designated HJR-1. (A detailed genetic study of this and other mutants obtained by this technique and a full description of the use of affinity chromatography to isolate CRM mutants of *D. discoideum* will be published elsewhere (J.R. *et al.*, in preparation).)

* Permanent address: Bose Institute, Calcutta, India.

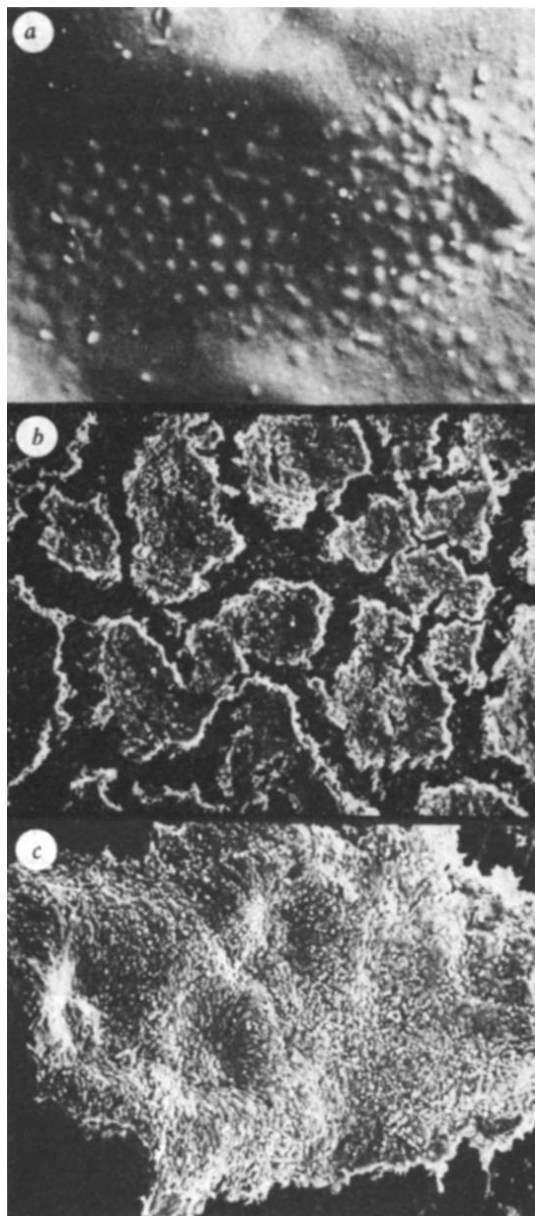


Fig. 1 Light microscopy of HJR-1 cells and comparative scanning electron microscopy of HJR-1 and NC-4 cells at the 6-h stage of development. For scanning electron microscopy, cells were fixed with glutaraldehyde, post-fixed with OsO_4 , dehydrated with an ethanol series and critical pointed dried. *a*, Light microscope picture of HJR-1 cells which had been allowed to develop for 16 h. *b*, *c*, Scanning electron micrographs of HJR-1 cells (*b*) (magnification $\times 150$) and NC-4 cells (*c*) at the 6 h stage of development (magnification $\times 150$).

Development of HJR-1 is blocked at the 3–6 h stage

The sequence of developmental events in NC-4 cells is as follows. When the source of nutrients is removed, the cells acquire sensitivity to cyclic AMP and move towards a region of higher cyclic AMP concentration. This process results in the entrainment of a large number of cells which, as they come together, become cohesive, resulting in the formation of loose and finally tight aggregates. Shortly before the onset of cohesion (after approximately 6 h of development), expression of CBPs on the cell surface begins. The tight aggregates are integrated into responsive structures resembling small slugs. Then, each organism differentiates into two discrete cell types (spores and stalks) and forms an erect fruiting body³¹.

In contrast to wild-type cells, strain HJR-1 does not proceed past the formation of loose aggregates. After the removal of the

nutrient source, HJR-1 cells move towards centres and form regularly spaced small mounds (Fig. 1*a*) characteristic of loose aggregates. The formation of loose aggregates by HJR-1 cells is confirmed by the scanning electron micrograph (Fig. 1*b*), which at higher magnification shows that these aggregates are made up of single cells in loose contact with each other (not shown). NC-4 cells, on the other hand, form large, tight aggregates in which the individual cells are in close contact (Fig. 1*c*). As chemotaxis and cell streaming are prerequisites for the formation of regularly spaced loose aggregates, we conclude that these processes are not altered in HJR-1 cells and that the development of HJR-1 cells is blocked between 3 h (onset of the formation of loose aggregates) and 6 h (formation of tight aggregates).

Siu *et al.*³² have reported that there is an accumulation and specific removal of certain plasma membrane proteins at discrete times of development. By using mutants blocked at specific points of development, they have also linked the appearance and disappearance of several membrane proteins to specific morphogenetic events. To study further the step at which the developmental sequence in HJR-1 is blocked, plasma membranes from wild-type NC-4 and mutant HJR-1 cells at different developmental stages were prepared³³ and their protein profiles compared after separation of the proteins in a 7–15% SDS-acrylamide slab gel³⁴. As with the morphological studies, the protein profiles of wild-type and mutant plasma membranes are consistent with a block at 3–6 h of development (data not shown).

HJR-1 cells are non-cohesive and lack haemagglutination activity

The cohesiveness of NC-4 and HJR-1 cells was measured by the standard assay³⁵. Microscopic observation revealed that the NC-4 cells re-form into large, tight aggregates within 5 min of the dispersion of the cell suspension, whereas HJR-1 cells remain mostly as single cells. As is typically found in this type of experiment, 48% of NC-4 cells are left as single cells after 5 min of dispersion of the cell suspension. These values fall to 45% within 15 min and remain constant up to 30 min. In the case of HJR-1 cells, 99% of the cells remained as single cells after 5 min and 95% of the cells were single cells up to 30 min (Fig. 2).

The endogenous CBPs of *D. discoideum* cells are defined by their ability to bind to galactose ligands and the agglutination of sheep red blood cells by one of them (CBP-26). For the interpretation of data to be presented later, it is important to note that although both CBP-26 and CBP-24 bind galactose-containing ligands, only CBP-26 agglutinates sheep red blood

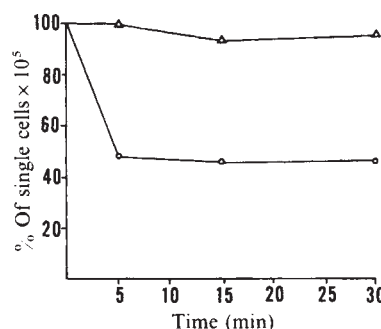


Fig. 2 Cohesiveness of NC-4 and HJR-1 cells. NC-4 and HJR-1 cells were allowed to develop for 16 h in the absence of bacteria on Whatman no. 50 filter paper resting on pads saturated with buffered salt solutions³¹. The cells were taken up in 20 mM EDTA in 17 mM phosphate buffer, pH 6.4. Cell suspensions were dispersed into single cells by pipetting in and out of a 10-ml pipette. The cell concentration was adjusted to 5×10^6 cells ml^{-1} in 10 mM EDTA in 17 mM phosphate buffer, pH 6.4. Aliquots (400 μl) of cell suspensions were dispersed into individually cut out wells of a Linbro multi-well test plate. The wells were gyrated at 150 r.p.m. in a G-10 Gyrotory shaker at 22 °C. Samples were taken at specified times and the single cells counted in a haemocytometer. Cohesiveness is expressed as the per cent of single cells remaining in the suspension after the formation of the aggregates. For the zero time point the cells were adjusted to the correct densities and the number of single cells was counted in the haemocytometer.

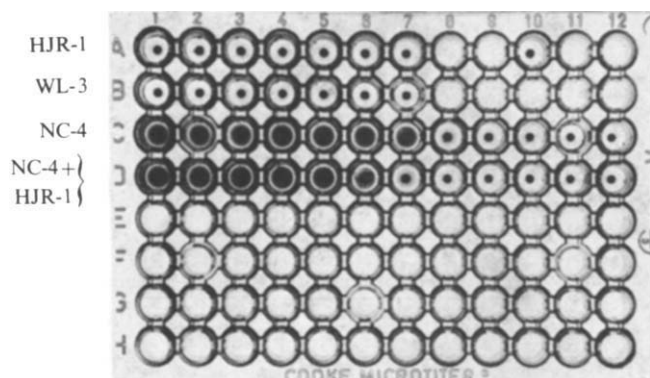


Fig. 3 Haemagglutination assay of NC-4, HJR-1 and WL-3 cell lysates. The soluble protein fractions of NC-4, HJR-1 and WL-3 cells at the 6-h stage of development were tested for haemagglutination activity against formalinised sheep erythrocytes²⁵. The protein concentrations were determined by the Lowry method⁴⁰. The starting concentrations of NC-4, HJR-1 and WL-3 were adjusted to the same amount. To assay the mixture, the soluble protein fractions of NC-4 and HJR-1 were mixed in equal amounts. The end point of titration was taken as the first dilution of the extract at which the treated cell lysate formed a clearly circumscribed dot at the bottom of the plate well. The agglutination activity of an extract is defined as the reciprocal of the end point dilution.

cells²⁸. Therefore, as an initial screen we tested for sheep red blood cell agglutination by our mutant. The soluble protein fractions from wild-type NC-4 cells and HJR-1 cells were tested for haemagglutination activity against formalinised sheep erythrocytes (Fig. 3 and ref. 25). As a standard, a non-cohesive mutant, WL-3, which has very little CBP, was included in our studies. At an initial protein concentration of 1.2 mg ml^{-1} , HJR-1 and WL-3 showed no agglutination of sheep red blood cells but NC-4 gave a titre of 1:128. To check for the presence of an inhibitor of agglutination in the HJR-1 protein fraction, the soluble protein fractions of NC-4 and HJR-1 were mixed in equal amounts and tested for haemagglutination. The mixture gave an agglutination titre of 1:64 (Fig. 3). This indicates that no inhibitor is present in the HJR-1 cell fractions and their inability to agglutinate the sheep erythrocytes is due either to the absence or inactivity of CBP-26.

CBP-26 is present in HJR-1 but is not functional

The above studies show that HJR-1 does not contain functional CBP-26. We then used a radioimmunoassay to quantitate the amount of CBP present in HJR-1 and NC-4 cells. As a control for specificity of assay, the non-cohesive mutant, WL-3, and wild-type NC-4 cells, were included in the study. In each case soluble protein fractions from cells which had been allowed to develop for 16 h were studied. Table 1 gives the concentrations of CBP in the soluble protein fraction, the amount of CBP per cell and the molecules of CBP per cell at 16 h of development for NC-4, HJR-1 and WL-3 cells. The results show that although CBP-26 does not function in HJR-1 cells, a comparable amount of protein is present. As radioimmunoassays only measure cross-reacting material and give no measure of the molecular nature of the antigens, we next characterised the molecular size and immunological relatedness of the CBP subunits in wild-type and mutant cells. For the former study, the IgG fraction of antibody reactive to both CBP-24 and CBP-26 was coupled to Sepharose 4B. The results in Fig. 4 show that molecules with molecular sizes of 26,000 and 24,000 were present in both the wild-type and mutant cells. The immunological relatedness of CBP molecules in the two types of cells was then determined by Ouchterlony double diffusion (Fig. 5), which showed that they have immunologically identical CBP-26 and CBP-24.

As we found CBP-26 in the soluble protein fractions of HJR-1 cells, we next determined if CBP was present on the

external surface of the plasma membrane of these cells, as has been reported for wild-type cells²⁶. NC-4 and HJR-1 cells at the 12-h stage of development were selectively radioiodinated by the lactoperoxidase method and the CBP was precipitated with antibody²⁶. The presence of ^{125}I radioactivity in the precipitate would indicate the association of CBP with the external surface of the plasma membrane. Figure 6 shows that when such a precipitate was solubilised and reduced for SDS-polyacrylamide gel electrophoresis, a major peak appeared for both NC-4 and HJR-1 in the region of 26,000 molecular weight. This protein was not present in the precipitate when normal goat serum was substituted for antibody against CBP. Thus, the block in HJR-1 does not seem to be an inability to insert CBP-26 into the plasma membrane.

The immunological studies showed the presence of CBP-26 in HJR-1. However, the soluble protein fractions of HJR-1 cannot

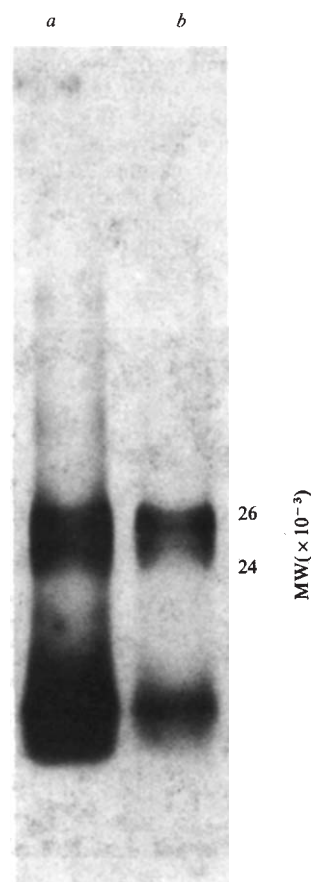


Fig. 4 Autoradiograph of CBP subunits isolated from 16-h cell lysates of HJR-1 and NC-4 by immuno-affinity chromatography. Purified goat anti-CBP IgG was coupled to epichlorohydrin-cross-linked Sepharose 4B (ref. 42). The cell lysate of NC-4 and HJR-1 (equivalent to 1×10^8 cells) was loaded onto a 1-ml column (6 mg of goat anti-CBP IgG per ml of Sepharose 4B) which had been equilibrated with PBS containing 0.4 M galactose. The beads had been treated with 2 M neutral trichloroacetic acid (TCA) before packing the column. The columns were washed with PBS-0.4 M galactose and then with 0.5 M neutral TCA, and the adsorbed proteins were eluted with 2 M neutral TCA. The column eluates were dialysed against PBS at 4 °C and the proteins were labelled with ^{125}I by the chloramine T method of McConahey and Dixon⁴³. The labelled proteins were immunoprecipitated with goat anti-CBP serum. The precipitates were solubilised and reduced by heating for 5 min at 90 °C in 1% SDS, 8 M urea and 2% β -mercaptoethanol. The reduced material was analysed by electrophoresis in 7–15% gradient slab gel prepared according to the method of Laemmli³⁴. The stacking gel was 3% acrylamide. Electrophoresis was carried out at a constant current of 20 mA for 3.5–4 h. The gel was fixed and stained in a solution of 25% isopropanol, 10% acetic acid and 0.5% Coomassie brilliant blue for 3 h. Destaining was achieved by washing the gel in a solution containing 10% methanol and 7% acetic acid. For autoradiography the gel was dried down onto a piece of paper and then placed in direct contact with Kodak RPX-OMAT film in a light-proof box. a, HJR-1; b, NC-4.

Table 1 Quantitation of CBP-26 in different strains of *D. discoideum*

Strain	Cell cohesion	Concentration of CBP-26 (ng per µg of soluble protein)	ng of CBP-26 per cell	Molecules of CBP-26 per cell
NC-4	+	7	1.4×10^{-4}	8.4×10^5
HJR-1	-	5	1.0×10^{-4}	6.0×10^5
WL-3	-	0.45	0.09×10^{-4}	0.5×10^5

Radioimmunoassay was done by the procedure previously described by Siu *et al.*²⁶ To construct a standard curve, purified CBP-26 was used. To quantitate the amount of cytoplasmic CBP in NC-4, HJR-1 and WL-3 cells, the cells were allowed to develop for 16 h and then collected for assay. Protein concentrations of each sample were determined by the method of Lowry *et al.*⁴⁰

agglutinate sheep erythrocytes. As, in wild-type cells, both CBP-26 and CBP-24 can bind to the galactose matrix of Sepharose, we were able to test whether the defect in this mutant was due to an altered binding affinity of CBP-26 for galactose. The soluble protein fractions of NC-4 and HJR-1 cells were subjected to affinity chromatography on Sepharose 4B gels. After washing the column with phosphate-buffered saline (PBS), the bound material was eluted with 0.4 M galactose. On polyacrylamide gel electrophoresis (Fig. 7), the galactose binding proteins isolated from wild-type NC-4 cells appear as two protein bands of molecular weights 26,000 and 24,000, the former being the major protein band. However, HJR-1 gives only one protein band of molecular size 24,000. This protein is the subunit of the other CBP, CBP-24, and here its presence

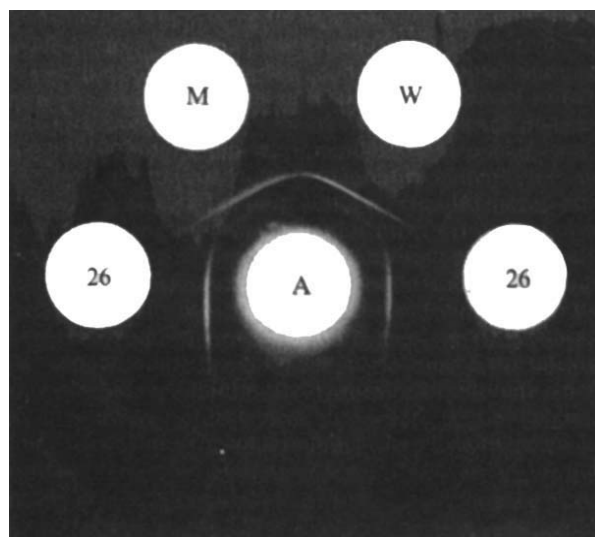


Fig. 5 Immunological characterisation of NC-4 and HJR-1 cell lysates by Ouchterlony analysis. Ouchterlony plates were made with 1% agarose impregnated with 0.4 M D-galactose, 0.15 M NaCl. The centre cell, marked A, contains 10 µl of goat antibody to both CBP-24 and CBP-26. Peripheral wells marked M and W contained, respectively 10 µl of soluble protein fractions of HJR-1 and NC-4 cells at the 16-h stage of development. The wells marked 26 contained 10 µl of purified CBP-26.

serves as an internal control for the efficiency of our column. Together with the quantitative radioimmunoassay studies these affinity studies indicate that HJR-1 can synthesise CBP-26 and CBP-24, but that CBP-26 is altered and cannot bind to galactose-containing ligands. We therefore conclude that the mutant HJR-1 belongs to the cross-reacting material (CRM) class of mutants and probably has a defect in the structural gene for CBP-26. We further conclude that CBP-26 is essential for cell cohesion and aggregation in *D. discoideum* cells, and, as HJR-1 cells have functional CBP-24, it seems that this CBP alone is not sufficient for cohesion.

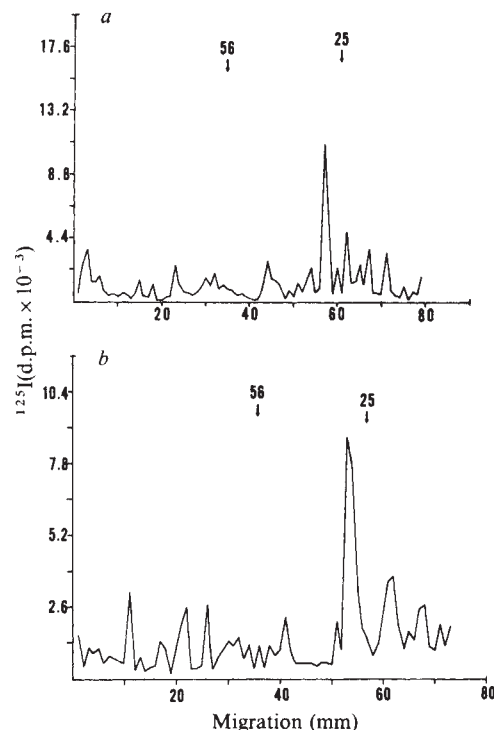


Fig. 6 Gel profile of an anti-CBP immunoprecipitate of ¹²⁵I-labelled surface proteins from developing cells of NC-4 (a) and HJR-1 (b). Cells were grown on *K. aerogenes* and developed on Whatman no 50 filter papers saturated with buffered salt solutions⁴¹. Developing cells at the 12-h stage were collected and their surfaces selectively radioiodinated by the lactoperoxidase method²⁶. Cells were lysed in 0.5% NP40 in the presence of 0.01% phenylmethylsulphonyl fluoride and the 100,000g supernatant solution was precipitated with goat anti-CBP serum, which in turn was precipitated with rabbit anti-goat IgG serum. The immunoprecipitate was solubilised and reduced by heating for 5 min at 90 °C in 10% SDS, 8 M urea and 2% β-mercaptoethanol, and analysed by SDS-polyacrylamide (10%) gel electrophoresis for 4 h at 3 mA per gel. ¹²⁵I-label and goat IgG were added in each gel as protein markers. Frozen gels were sectioned into 1-mm slices using a Joyce-Loebl automatic gel slicer, and the radioactivity was counted in a nuclear Chicago automatic γ-radiation counter. Data were corrected for crossover and efficiency of isotope detection. No peaks appeared in either case when normal goat serum was substituted for goat anti-CBP serum. The data are plotted on different scales in the two cases.

Agglutination activity of CBP-26 and cell cohesion are always parallel in revertants

To test the possible correlation between the defect in CBP-26 and the inability to form tight aggregates, we isolated revertants of HJR-1 that had regained the ability to aggregate and measured their haemagglutination activity. Exponentially growing cells of HJR-1 were mutagenised with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NMG)³⁶, 0.1–1% survival, and revertants were identified by their ability to aggregate and culminate. Such revertants arose at a frequency (1.1×10^{-3}) characteristic of NMG-induced point mutations in *D. discoideum*³¹. The 58 revertants fell into three roughly equal classes (Table 2). Class 1 revertants displayed wild-type aggregation behaviour in that they formed an equal number of similarly sized slugs and sori as NC-4. Class 2 revertants formed slightly fewer (~25% of wild type) and smaller slugs than NC-4 or the class 1 revertants. Class 3 revertants formed only a few small slugs and sori.

If CBP-26 were involved in determining the aggregation behaviour of these strains, one should be able to correlate the CBP-26 activity with the aggregation behaviour. We tested this by measuring the haemagglutination activity of several strains from each class of revertants (Table 2). As expected, class 1 revertants displayed wild-type haemagglutination activity; class

Table 2 Agglutination activity in revertants of HJR-1

Class	No. of revertants found	No. of strains tested	Greatest dilution that produced agglutination*
Mutant	—	1(HJR-1)	<1/1
Wild-type	—	1(NC-4)	1/256–1/512
1	18	5	1/256–1/572
2	15	4	1/128
3	25	5	1/16–1/32

* The 100,00g supernatants from 16-h cells of each strain were diluted to a final protein concentration of 2.0 mg ml⁻¹. These samples were then analysed for sheep erythrocyte agglutination activity as described in Fig. 3.

2 revertants displayed 25–50% wild-type activity and class 3 revertants had little activity. Thus, there is a strong correlation between haemagglutination activity (CBP-26 lectin activity) and the ability to aggregate, indicating that CBP-26 and its lectin-like activity are intimately involved in aggregation.

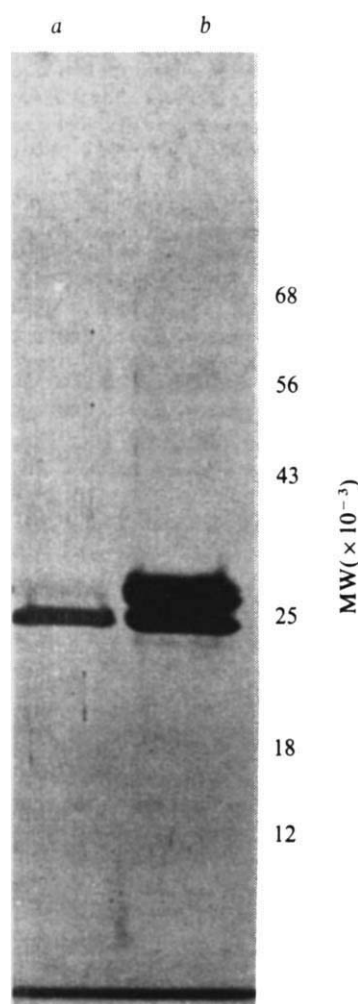


Fig. 7 Electrophoretogram of the galactose-binding CBP subunits isolated from the soluble protein fractions of NC-4 and HJR-1 16-h cells. The cell lysate was prepared according to the method of Siu *et al.*²⁶. NC-4 and HJR-1 cell lysates, equivalent to 5×10^8 cells were slowly loaded onto a 5-ml Sepharose 4B column which had been equilibrated with PBS. The column was washed with PBS at a flow rate of 1 ml min⁻¹ until the absorbance of the effluent was low. The adsorbed materials were then eluted with 0.4 M D-galactose in PBS. The materials eluted with sugar were dialysed against water and lyophilised. The lyophilised samples were solubilised and reduced by heating for 5 min at 90 °C in 1% SDS, 8 M urea and 2% β -mercaptoethanol. The reduced materials were analysed by electrophoresis in a 7–15% gradient slab gel³⁴. The gels were stained and destained according to the method described in Fig. 4. a, HJR-1; b, NC-4.

Mutant HJR-1 cells synergise with wild-type NC-4 cells

The presence of wild-type cells in a population of unaggregated cells will often result in differentiation of the mutant cells and formation of viable spores of both genotypes (synergy)³⁷.

When mutants with precisely defined alterations in cell surface molecules are used, cell synergy studies can give important information on the nature and number of molecules involved in cell–cell interaction. As we know that HJR-1 cells have a defective CBP-26 it was especially interesting to carry out synergy studies on this mutant. Washed NC-4 and HJR-1 suspensions were mixed in appropriate dilutions so as to provide a range of population ratios, and spores from synergistically formed fruiting bodies were assayed clonally. The plaques were scored for properties of either the mutant (unaggregated plaques) or the wild type (plaques with slugs and fruiting bodies). Total plaque counts revealed a normal percentage of viability among the spores (efficiency ~54%). Table 3 shows the analysis of the spores from synergistically formed fruiting bodies; the presence of wild-type cells in the mixture allowed the mutant to develop.

The proportion of NC-4 and HJR-1 cells present generally determined the proportion of the two spore types in the fruiting bodies. For example, a ratio of four times NC-4 to one times HJR-1 allowed the appearance of only 12% HJR-1 spores. But, when the ratio of NC-4 to HJR-1 was changed to 1:4, the percentage of HJR-1 spores rose to 74%. However, as the ratio of HJR-1 to NC-4 was further increased, the number of sori formed declined. When the ratio of NC-4 to HJR-1 was 1:10, the ratio of NC-4 to HJR-1 in the sori was also 1:10. But as the ratio of NC-4 to HJR-1 was increased from 1:20 to 1:100, the ratio of NC-4 to HJR-1 in the sori remained about 1:20 (Table 3).

Treatment of spores with Triton X-100 to destroy any HJR-1 amoebae which might have contaminated the spores did not decrease the percentage of mutant plaques.

Discussion

The above results indicate that one of the CBPs (CBP-26) has a critical role in cell–cell cohesion and development. The role of CBP-24 is less certain although as it is present in HJR-1 we can say that by itself it cannot mediate cell cohesion. Perhaps CBP-24 is involved later in development. Apart from questions concerning the role of CBP-24 in development, its presence in our mutant confirms that HJR-1 is not simply a programme mutant. One of the difficulties in using mutants to define the role of proteins with developmentally regulated expressions is that the absence of a protein in a mutant can mean either that the

Table 3 Phenotypic analysis of spores from synergistic fruitings of NC-4 and HJR-1

Ratio of NC-4 to HJR-1	No. of clones examined	No. of wild-type clones	No. of mutant clones	Ratio of NC-4 to HJR-1 in the sori
4:1	203	178	25	7:1
1.5:1	163	110	53	2:1
1:1	83	44	39	1:1
1:1.5	121	61	60	1:1
1:4	200	52	148	1:3
1:10	320	26	294	1:11
1:20	241	12	229	1:19
1:50	214	11	203	1:19
1:70	362	18	344	1:19
1:100	389	16	373	1:23

Synergy assays were done according to Siu *et al.*⁴⁴. NC-4 and HJR-1 cells were mixed in equal numbers or in different ratios. Individual sori were picked up 45 h after the initiation of development. Spores were released by freezing and thawing the sori once in water and then 20 cells were plated per sm agar plate with *K. aerogenes*. Cells were allowed to grow at 22 °C until each clone was about 2–3 mm in diameter. HJR-1 clones gave rise to aggregateless plaques, whereas NC-4 clones gave rise to plaques with slugs and fruiting bodies. In some studies spores were treated with 0.2% Triton X-100 before plating.

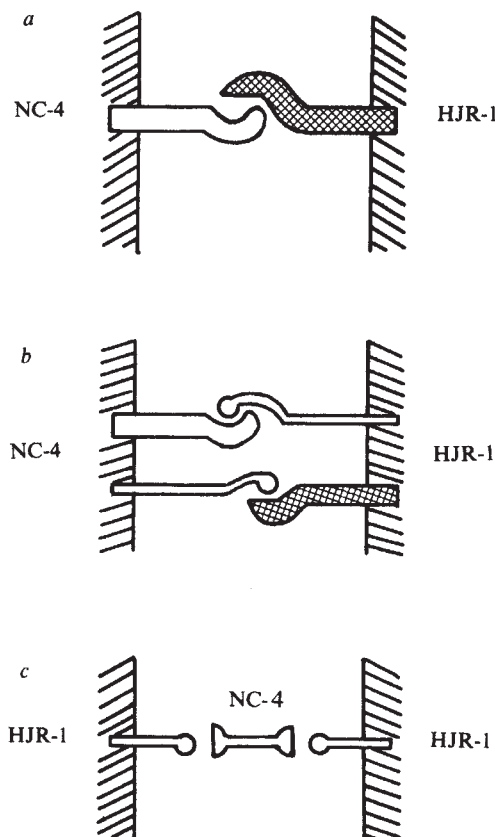


Fig. 8 Models for specific cell-cell interactions in *D. discoideum* cells. The defective CBP-26 is shaded. The carbohydrate moieties of the receptors are drawn as open circles. *a*, Trans-membrane dimer formation by protein-protein association between CBP-26 of adjacent cell surfaces. *b*, Dimer formation by interactions between complementary protein-carbohydrate molecules on opposing cell surfaces. *c*, The protein, CBP-26, acts as a cell ligand which is secreted into the intercellular spaces and binds the slime mould amoebae together by attaching to carbohydrate receptors on the adjacent cells.

protein is important to development or simply that the developmental programme has not proceeded far enough for the protein to be expressed. The latter mutants are termed programme mutants and are of little use in determining the role of proteins. As CBP-26 and CBP-24 are expressed at approximately the same time in development, the presence of CBP-24 indicates that HJR-1 can proceed far enough into development to express CBP-26.

In terms of the general problem of cell-cell cohesion in eukaryotic development, the most important aspects of the data presented here are the synergy studies. Because wild-type cells completely complement the mutant for development one must envisage a lectin-ligand or bi-molecular system. This is illustrated in Fig. 8, which shows several models for the molecular

basis of cell-cell cohesion. A like-like protein interaction (Fig. 8a) seems to be ruled out by our synergy studies. An open possibility is that during synergy, wild-type cells secrete enough CBP into the intercellular matrix to bind to the receptor of mutant cells and carry them through development (Fig. 8c). We have carried out some experiments to test this possibility (J.R., unpublished). We found that the development of HJR-1 cells is not helped by the presence of wild-type NC-4 cells when the two cell populations are separated by Millipore filters. Thus, these experiments do not support the possibility that CBP or any diffusible factor is secreted into the medium. Furthermore, Rosen *et al.*²⁵ have reported that CBP is not secreted into the medium during normal cellular development of wild-type cells. If we rule out a secreted molecule, the proportional development of mutant and wild-type cells also means that during synergy one wild-type cell can complement as many as 20 HJR-1 cells during aggregation. It can be calculated that one perfectly spherical cell can accommodate 12 other spherical cells around it by contact³⁸. As the NC-4 cells are not spherical, and extend pseudopodia, it is not unreasonable to assume that 20 mutant cells could be contacted by one wild type. Thus, we feel that the known ability of CBP to bind to carbohydrate ligands plus the synergy data support the model shown in Fig. 8b. The simplest explanation is that during development CBP binds to a carbohydrate receptor, and although our mutant lacks CBP, it has the receptor and thus can be complemented by wild-type cells which contain CBP. Aside from models of cell-cell interactions, the fact that HJR-1 can be complemented by wild-type cells in a synergy study shows that except for the defect in CBP-26, the cells are completely competent to adhere, aggregate and proceed through the life cycle.

The fact that the HJR-1 mutant is of the CRM class suggests that there is a lesion in the gene encoding CBP-26. There are, of course, alternative models; the product of the CBP-26 gene might require modification before it can become functional and the mutation could be in the gene encoding the modifying function. Unlike CBPs or lectins from other systems, CBP-26 is not glycosylated and thus this common modification is not important here. However, we cannot rule out other protein modifications such as methylation or acetylation. The most direct approach to ensure that the defect is in the structural gene for CBP-26 is to map the gene rather than to map the protein. We are now trying to clone the gene for CBP-26 from wild-type and HJR-1 cells.

Müller and Gerisch have shown that Fab antibody fragments against a glycoprotein with a molecular size of 80,000 daltons blocks cellular adhesion³⁹. This glycoprotein is distinct from both the CBP and the CBP receptor²⁴. Taken together, these results and our own data indicate that more than one molecular system is necessary for proper cell cohesion and development.

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Identification of the UV nightglow from Venus

THE presence of atmospheric ultraviolet (UV) emission between 1,350 and 2,200 Å from the nightside of Venus was first detected by instruments on board the Mariner 5 flyby¹. Recently this emission was observed by the UV spectrometer on Pioneer Venus Orbiter at 2,068 Å and identified by Stewart *et al.*² as the (0, 0) Cameron band ($a^3\Pi-X^1\Sigma$) of CO. Their interpretation suggested a significant input of energy into the night-side thermosphere from the interaction of the solar wind with the planet. We report here the preliminary results of observations of the nightside spectrum of Venus made with the International Ultraviolet Explorer (IUE) observatory when Venus was near greatest elongation in January 1979. Three features are clearly seen in the spectra, and the spectral resolution of the instrument is adequate to identify them as bands of the nitric oxide ($\delta(C^2\Pi \rightarrow X^2\Pi)$) system rather than as Cameron bands. The δ -bands, present in the Earth's twilight and night time airglow spectra³, are produced by the radiative association of nitrogen and oxygen atoms and indicate a significant population of atomic nitrogen at or below the Venus turbopause.

The IUE satellite and its application for planetary observations have been described previously⁴. The high visual brightness of the planet completely saturates the IUE acquisition monitor (fine error sensor, FES) so that it is not possible to point at and track Venus in the conventional manner. Rather, it is necessary to use a technique in which the illuminated portion of the planet is centred on the large spectrograph slit (roughly elliptical $10'' \times 20''$) by minimising the total amount of light reflected from the slit jaw to the FES. At the time of our observations on 11 and 12 January 1979, the disk was 46% illuminated and the semi-diameter of Venus was $13.6''$ so that when centred on the large slit most of the radiation entered the spectrograph and only a small fraction was available to the FES. To obtain spectra of the dark side of the planet, the large slit was offset by dead reckoning in the anti-sunward direction by $15''$ and by $20''$; the long dimension of the slit remained nearly parallel to the terminator. The larger offset was used in an attempt to reduce the effect of instrumentally scattered light in the telescope from the illuminated side of Venus.

Low dispersion spectra of the dark side of Venus were obtained with both spectrographs. A portion of the short wavelength exposure is shown in Fig. 1. The (0, 0) band of the NO δ -system at 1,909 Å is clearly present, and the $\delta(0, 1)$ band at 1,980 Å is indicated, superimposed on instrumentally scattered continuum from the dayside. The (2, 0) Cameron band of CO at 1,928 Å does not seem to be present, but some care must be taken as this wavelength is partially contaminated by a reseau

mark on the camera face. The wavelength uncertainty is ± 2.7 Å, so that the possibility that the $\delta(0, 0)$ band is misidentified and is in fact the (2, 0) Cameron band seems unlikely. Verification of wavelength position to this accuracy is made with the aid of the $H\text{I } \text{L}\alpha$ line at 1,216 Å while the dispersion constant is determined from the spectrum of an on-board reference lamp. Note that these bands do not appear in the dayside spectrum which seems to contain only reflected solar radiation as determined from a short exposure made immediately after the exposure shown in Fig. 1.

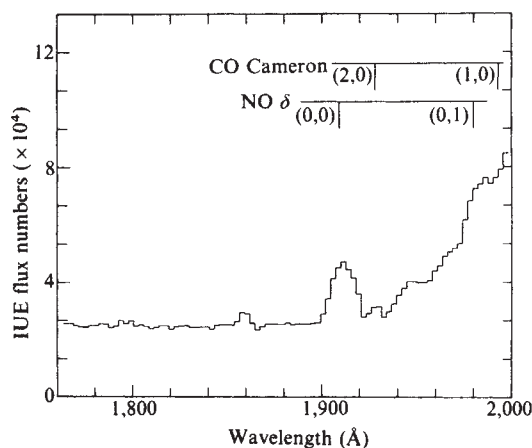


Fig. 1 A portion of the spectrum of the darkside of Venus obtained from a 30-min exposure with the short-wavelength spectrograph (SWP 3878). Both the underlying continuum and the steep increase near 2,000 Å are due to instrumentally scattered light. The positions of the CO Cameron and NO δ -bands are indicated. The response of the spectrograph is nearly uniform in this wavelength region.

The $\delta(0, 1)$ band is more clearly seen, along with the $\delta(0, 2)$ band at 2,055 Å in the long wavelength spectrum shown in Fig. 2. Again the positions of the bands agree with the expected wavelengths of the NO δ -bands to within the experimental uncertainty of ± 4.5 Å. In this spectrum the instrumentally scattered light becomes more severe with increasing wavelength but, nevertheless, the data of Fig. 2 clearly show the emission features superimposed on the scattered light. In the future it should be possible to remove most of the scattered continuum by computer analysis of the spatial distribution of light across the image of the slit.

Despite the presence of a strong underlying continuum of scattered light, the relative band intensities shown in Figs 1 and 2 seem to be the same as those observed both in the Earth's airglow³ and in the laboratory⁵, where the excitation mechanism has been identified as radiative association of oxygen and nitrogen atoms. Bands from $v' \geq 1$ of the NO C²Π state are not present in the chemiluminescent spectra as these vibrational levels are rapidly depopulated by predissociation. Our interpretation is consistent with the Pioneer Venus Orbiter observation² as the $\delta(0,2)$ band at 2,055 Å would be partially transmitted by the 13 Å spectral bandwidth of that instrument when centred near 2,068 Å. From the short wavelength spectrum of Fig. 1, assuming the Venus darkside to uniformly fill the 10" × 20" aperture, we deduce a column emission rate of 0.5 kR for the $\delta(0,0)$ band. This is a lower limit, as precise pointing information from the FES is not available and it is possible that the spectrograph aperture extended beyond the planet's limb. Despite this uncertainty, the brightness of the observed δ -bands is consistent with the 1–2 kR for the disk brightness (away from the limb) reported by Stewart *et al.*².

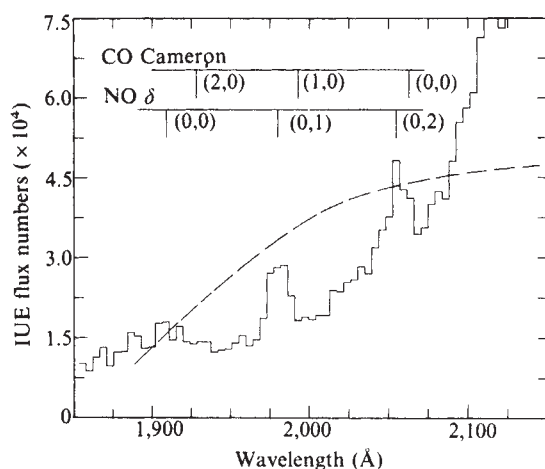


Fig. 2 As for Fig. 1, but from a 20-min long-wavelength exposure (LWR 3470). The dashed line gives the relative instrument response as a function of wavelength.

In the Earth's thermosphere atomic nitrogen is produced primarily by solar EUV dissociation of N₂, but the N abundance and altitude distribution are strongly controlled by atmospheric chemistry⁶. Shortly after sunset the relative abundance of N to O is $\sim 10^{-3}$ in the altitude range where the chemiluminescent reaction occurs and the total vertical column emission rate of the $\delta(0,0)$ band is ~ 5 R. On Venus the emission rate is at least a factor of 100 greater implying either a larger N:O ratio or a much greater column abundance of atomic oxygen. Evidence for the latter was previously given by the Venera 9 and 10 observations of the O₂ Herzberg II bands in the visible nightglow⁷ which are produced by the three body reaction of O + O + CO₂ and a large relative abundance of atomic oxygen near the evening terminator has been reported by the Pioneer Venus mass spectrometer experiments⁸. In addition the mass spectrometer data show a N₂:O ratio of $\sim 1:3$ near 150 km so that a sufficient source of nitrogen atoms is present in the dayside thermosphere. However, as both O and N are depleted on the nightside through various recombination mechanisms, their presence on the darkside indicates efficient transport of atoms from the dayside where they are produced. Further analysis, using more data expected from Pioneer Venus Orbiter, should permit a quantitative analysis of the odd nitrogen chemistry in the upper atmosphere of Venus. This, in turn, should lead to a better understanding of the transport of dissociation products from the dayside to the nightside of the planet.

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P. D. FELDMAN
H. W. MOOS
J. T. CLARKE

Physics Department,
Johns Hopkins University,
Baltimore, Maryland 21218

A. L. LANE

Jet Propulsion Laboratory,
Pasadena, California 91103

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A two-dimensional calculation including atmospheric carbon dioxide and stratospheric ozone

INCREASED use of fossil fuels¹ and deforestation² have led to enhanced levels of CO₂ in the atmosphere with an increase of about 10% since the beginning of this century¹. This upward trend is continuing and it has been predicted that the concentration may double in less than 100 years^{3–5}. We describe here a calculation of the subsequent effect of increased CO₂ on stratospheric ozone, using a two-dimensional dynamical model of the atmosphere including radiative and photochemical processes.

As CO₂ plays a major part in the radiation balance of the troposphere and stratosphere, changes in its mixing ratio could have important climatic consequences and this has been considered in studies of the global circulation⁶. However, it is only recently that the subsequent impact of changes in CO₂ on atmospheric ozone has been considered. Luther *et al.*⁷, Boughner⁸ and Groves *et al.*⁹, using one-dimensional models, predict increases in ozone in the upper stratosphere associated with a

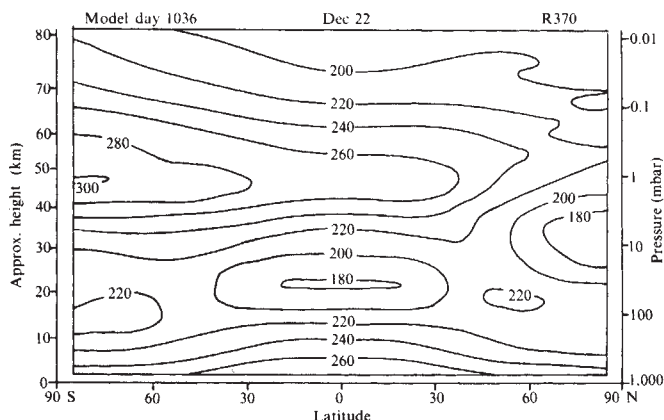


Fig. 1 The cross-section of temperature (K) from the run with the CO₂ mixing ratio at 320 p.p.m.

decrease in temperature there due to the enhanced long wave cooling by CO_2 . One-dimensional models include detailed descriptions of photochemical and radiative processes at the expense of a rather poor treatment of transport. With double the present CO_2 levels these models predict increases in the total column of ozone of between 1 and 5%.

One-dimensional models have serious limitations in perturbation studies as indicated by Groves *et al.*⁹ as many important processes are not included, and ideally a fully interactive three-dimensional calculation should be made. Such a three-dimensional calculation would, however, be very expensive computationally. The two-dimensional calculation presented here includes the feedback of changes in temperature on the mean circulation, but not on eddy transport, and allows latitudinal and seasonal effects to be studied. Using this model, one of us (J.A.P.)^{10,11} demonstrated the importance of the latitudinal and seasonal variations, omitted in one-dimensional models, when considering possible perturbations to the ozone layer by fluorocarbons.

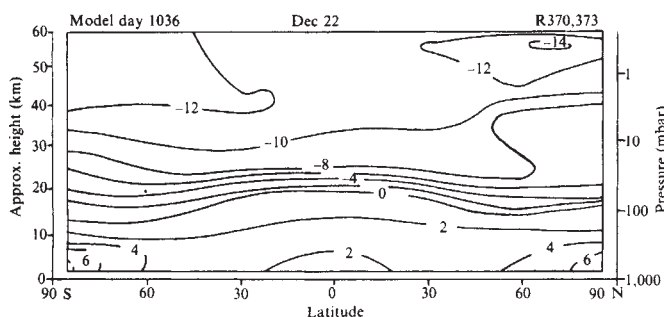


Fig. 2 The cross-section of the difference in temperature (K) following the doubling of the CO_2 from 320 to 640 p.p.m.

The two-dimensional model is described in detail in refs 12 and 13. However, we will briefly describe some significant changes in the radiative and photochemical schemes. A detailed treatment of photochemical processes is included (see ref. 10), the continuity equation being solved for the following species or groups of species: $\text{O}(^1\text{D})$, O and O_3 ; NO , NO_2 and N ; HNO_3 ; H , OH and HO_2 ; H_2O_2 .

The cooling-to-space approximation for long-wave cooling by CO_2 has been replaced by a more detailed treatment in which the exchange between different layers and the breakdown of local thermodynamic equilibrium are included. Following Curtis¹⁴⁻¹⁶ the heating rate profile $\mathbf{H}$, at each model level is related to the temperature or Planck function profile, $\mathbf{B}$, by $\mathbf{H} = \mathbf{CB}$. The matrix $\mathbf{C}$ describes the atmospheric transmission between different levels, which for a uniformly mixed gas such as CO_2 needs to be calculated just once assuming that the transmission is not strongly dependent on the temperature, and allows for the deviation from local thermodynamic equilibrium.

The cooling-to-space approximation is retained for the $9.6 \mu\text{m}$ O_3 band, with the cooling dependent only on changes in the temperature and not on changes in the ozone concentration. The major long-wave cooling in the stratosphere and mesosphere is due to CO_2 so that this simplified treatment of the ozone cooling should not introduce serious error.

The constant radiative cooling in the troposphere has not been changed. However, in the experiment with doubled CO_2 , the model sea-surface temperatures were modified, based on the results of Manabe and Wetherald⁶. The tropospheric temperatures can then be altered by the convective adjustment scheme. The lower stratosphere, below about 24 km, is assumed to be in radiative equilibrium as in our previous calculations. We offer an *a posteriori* justification for these approximations. The agreement with the calculations of Manabe and Wetherald in the lower atmosphere is reasonable and our main interest is in the behaviour of the stratosphere.

Two calculations have been performed. In the control run a CO_2 mixing ratio of 320 p.p.m. was used in the radiation calculations, appropriate roughly to present day levels, and in the second run the mixing ratio was doubled, corresponding to a year around 2030^{5,9,17}. Figure 1 shows the cross-section of temperature at the December solstice from the control run. Note that the improved radiative and photochemical schemes have resulted in a more realistic representation of the stratospheric temperatures than in previous versions of the model¹². The equatorial tropopause is, however, at too low a temperature and the cold summer mesopause is not reproduced.

Figure 2 shows the temperature difference between the control and the run with doubled CO_2 . The increase in tropospheric temperature agrees qualitatively well with the detailed calculations of Manabe and Wetherald⁶. In the stratosphere there are large reductions in the temperature due to increased long wave cooling. The stratopause temperatures are reduced by more than 10 K, which is similar to the one-dimensional model calculations of Luther *et al.*⁷ and Groves *et al.*⁹, and greater than that of Boughner⁸. Together with the change in the temperature structure there is a reduction in the strength of the jets in the middle stratosphere with both easterly and westerly maxima decreasing by about 10%.

Because of the temperature dependence of the ozone production and destruction rates, the ozone concentration in the stratosphere is inversely correlated with temperature¹⁸. The percentage change in the ozone concentration between the two runs is shown in Fig. 3 for the Northern Hemisphere winter solstice. In the upper stratosphere where the temperature has decreased, the ozone concentration increases. This increase in ozone gives rise to increased solar absorption and thus to a reduced penetration of UV radiation into the lower stratosphere. The photodissociation of molecular oxygen, and hence ozone production, is reduced, explaining the area of decreased ozone concentration found in the equatorial lower stratosphere. This is the reverse of the 'self healing' effect seen in the calculation of ozone perturbations due to fluorocarbons.

In the high-latitude lower stratosphere there is no decrease in the ozone concentration. One explanation is a reduced ozone destruction here, since below about 30 km in high latitudes the NO_2 concentration is less in the run with doubled CO_2 . This seems to be due to decreased production of odd nitrogen, $\text{O}(^1\text{D})$ being smaller in the lower stratosphere. However, dynamical effects will also be important in producing the modelled ozone changes; the problem is a complex one with dynamical, radiative and photochemical processes all playing a part.

The latitude-time section of the percentage increase in total ozone is shown in Fig. 4. There are large variations in the increase with both latitude and season, the largest increases occurring in high latitudes during the winter months. Smallest changes are found in equatorial latitudes where to some extent the upper stratospheric increases are compensated for by reductions in ozone at lower altitudes.

Interestingly, these changes are in exactly the opposite sense to that predicted for the effect of fluorocarbons on ozone^{10,11},

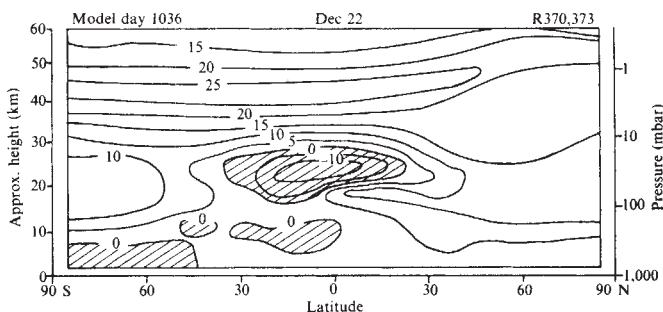


Fig. 3 The cross-section of the percentage difference in the ozone concentration following the doubling of CO_2 from 320 to 640 p.p.m. Areas of ozone decrease are shaded.

where the calculated reductions are largest in high latitudes in winter. This compensation was pointed out by Groves *et al.*⁹, who stressed that the two effects would not necessarily be additive. A calculation in which both perturbations are included seems to be necessary.

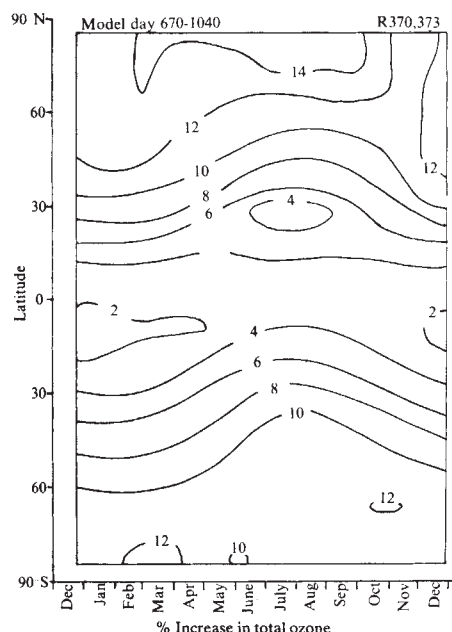


Fig. 4 The latitude-time section of the percentage increase in total ozone following the doubling of CO₂ from 320 to 640 p.p.m.

The total global increase in ozone for a doubling of CO₂ is calculated in this model to be about 7%, a larger value than found in the one-dimensional calculations. The changes in the upper stratosphere agree well with the results of Luther *et al.*⁷ and Groves *et al.*⁹ so that the discrepancies seem to be due to differences in the lower stratosphere. Two-dimensional models should describe the lower stratospheric ozone content better than one-dimensional models since in this region ozone is predominantly dynamically controlled. Note that in this calculation the CO₂ radiation calculations in the stratosphere were not changed below about 24 km, where the net radiative heating is, however, small. Clearly, further differences between this and the other calculations will lie in the precise radiative and photochemical schemes used.

In agreement with the previous one-dimensional calculations⁷⁻⁹ the model has calculated an increase in the ozone concentration of the upper stratosphere, associated with reductions in temperature there. One-dimensional models, however, do not include the coupling between the radiative sources and sinks and the circulation. The calculated changes in the stability of the stratosphere should certainly affect the vertical motion. Because of their inadequate treatment of transport processes, one-dimensional models are unable to represent accurately variations with latitude and season. Although this calculation has omitted many possible feedback processes, particularly in the troposphere where changes in albedo, in cloud cover and the hydrological cycle have been completely ignored, the model represents a significant advance beyond the one-dimensional calculations. The coupling between the radiative heating and the mean circulation has been included. Furthermore, latitudinal and temporal variations have been shown, unequivocally, to be important. This study, and earlier work on the effects of fluorocarbons^{10,11}, emphasises that latitudinal and seasonal effects need to be considered in the calculation of possible changes in stratospheric ozone. Furthermore, it is clear that dynamical, radiative and photochemical processes all play a part and should all be considered.

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J. D. HAIGH
J. A. PYLE

Department of Atmospheric Physics,
Clarendon Laboratory,
Oxford, UK

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The physical basis for absorption of light

THE effect of low intensity light on the wave functions of gas-phase molecules and atoms is well understood, and solutions of the time-dependent wave equation, or its equivalent, are common. However, two different operational approaches can be used to examine the relationship between the wave function and light absorption. The first approach¹ involves examining the time dependence of the populations of internal energy states. Changes in the average internal energy are then used to infer a corresponding absorption of energy from the radiant field by invoking energy conservation. The second approach² involves computing the polarisation of a medium induced by the radiant field. Absorption is then shown to be proportional to the imaginary part of the susceptibility. The latter is a natural approach for sample geometries which are small compared with the wavelength of the light, but it can also be used for larger

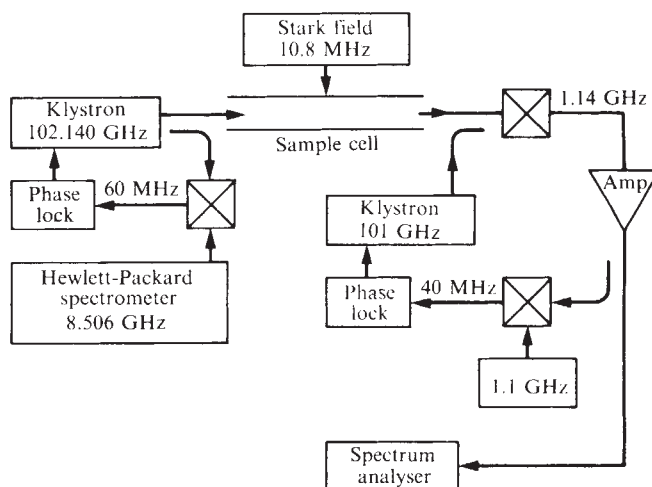


Fig. 1 Block diagram of the millimetre wavelength heterodyne spectrometer.

geometries and shorter wavelengths. When the sample geometry is large compared with the wavelength, the field from the polarisation lags behind the polarisation itself by 90° (ref. 3). Absorption then comes from the part of the induced polarisation which lags the incident field by 90° . Absorption of light in this picture is due to stimulated emission which is 180° out of phase with the incident field. Both the population and polarisation approaches have been used to describe experiments in which the absorption coefficient is observed as a function of time and as a function of the frequency of the incident light³. The two approaches are equivalent for the usual case in which a power detector is used to measure the time-dependent absorption coefficient in the sample⁴. In the experiment described here, a Stark-modulated sample is irradiated at a single frequency and radiation emerging from the sample is resolved into frequency components using a spectrum analyser to distinguish between the upper and lower modulation sidebands. It will be shown here that the upper and lower sidebands are not always equal and that this result is only consistent with a modified polarisation approach in which the stimulated emission is explicitly considered.

The experiment involves electric-field (Stark) modulation of the rotational absorption of methyl fluoride in the $K=1$, $J=2-1$ transition at 102,140.86 MHz. A block diagram of the millimetre wavelength heterodyne spectrometer is shown in Fig. 1. The source klystron was locked to the twelfth multiple of the output of an 8 GHz Hewlett Packard spectrometer. The local oscillator klystron was locked so that it was always 1,140 MHz lower in frequency than the source klystron. The heterodyne detector was a single silicon point-contact diode and produced output power into the spectrum analyser proportional to the product of local oscillator and signal oscillator powers. For the experiments described here, the methyl fluoride sample pressure was 80 mtorr, and an oscillating electric field at 10.8 MHz was supplied to the sample with a peak amplitude equal to 40 V cm^{-1} . The 102 GHz and 10.8 MHz fields were approximately parallel in the sample cell. In these conditions the $M = \pm 1$ transitions modulate linearly with the field up to a peak deviation of $\sim 14 \text{ MHz}$. The absorption linewidth at half maximum was $\sim 1.5 \text{ MHz}$.

Figure 2 shows tracings of the output from the spectrum analyser for three different settings of the signal frequency. For each trace the signal oscillator is fixed and what is displayed is the Fourier transform of the millimetre radiation field. The large signals correspond to the incident field, while the smaller signals are due to the modulation by methyl fluoride. (Small stray signals which did not disappear when the sample was pumped out have been suppressed for clarity.) When the signal oscillator is in resonance (curve *b*), the modulation sidebands are of equal intensity, as would be expected if the sample were amplitude modulating the incident power. Surprisingly, curves *a* and *c* show a different behaviour. For these scans, the signal oscillator was below or above the molecular resonance by one modulation frequency, and the sideband amplitudes are not at all consistent with amplitude modulation.

The theory of large-amplitude Stark modulation has been developed by Karplus for optically-thin samples in the absence of power saturation⁵. As the effect of the modulation appears in the time dependence of the absorption coefficient in ref. 5, the modulation is a form of amplitude modulation, and is not consistent with experiment. On the other hand, the induced polarisation can be obtained from equation (21) of ref. 5, and is proportional to the real part of the quantity

$$\sum_{s,n=-\infty}^{\infty} J_s(X) J_{s+n}(X) \exp[-2\pi i(\nu_c + n\nu_m t)] \times (\nu_m + \nu_0 - \nu_c - i/2\pi\tau)^{-1} \quad (1)$$

in which $J_s(X)$ is a Bessel function of order s with an argument equal to the peak Stark shift divided by the modulation frequency, ν_m ; ν_c is the incident frequency, ν_0 is the molecular resonance frequency, and τ is the relaxation time. The field

induced by this polarisation will have the same time dependence but will be shifted in phase by 90° . The $\pm M$ state symmetry results in a cancellation of the polarisation for terms in which n is odd. Therefore, the first side band of importance in equation (1) is for $n = \pm 2$. In conditions of this experiment, $2\pi\nu_m\tau = 0.14$ and $X = 1.3$. For curve *b* of Fig. 2, $s = 0$ is dominant, and the $n = \pm 2$ sideband amplitudes are proportional to $J_0(1.3) J_2(1.3) = 0.11$. For curve *a*, $s = -1$ dominates, and the $n = -2$ amplitude is proportional to $J_{-1}(1.3) J_{-3}(1.3) = 0.021$ while the $n = +2$ amplitude is proportional to $J_{-1}(1.3) J_1(1.3) = -0.272$. The explicit consideration of emission due to polarisation is thus qualitatively consistent with the experimental results. A more detailed quantitative comparison of experiment and theory in a variety of conditions will be covered elsewhere.

This experiment shows that light absorption should be more consistently described as a subtractive stimulated emission. The emission in a Stark modulation experiment is not consistent with amplitude modulation due to a modulated absorption coefficient. Any experiment involving time-dependent absorption

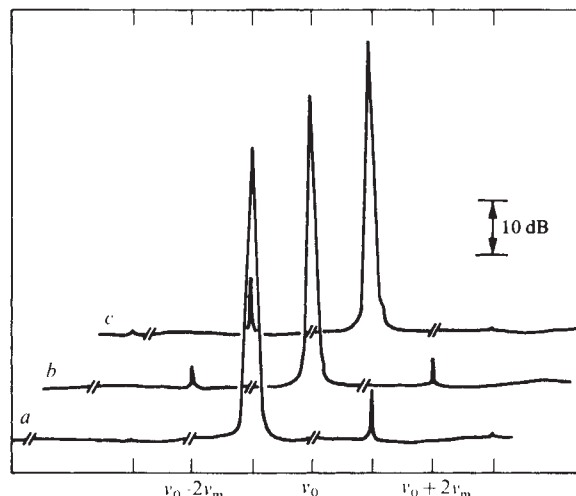


Fig. 2 Scans of the spectrum analyser for three values of the incident frequency. The horizontal frequency axis has been shifted so that the display corresponds to millimetre wavelength spectrum incident on the first mixer. The vertical relative power axis has been displaced 10 dB for clarity. Curve *b* was recorded when the incident frequency equalled the resonant frequency ($\nu_0 = 102,140.86 \text{ MHz}$). Curves *a* and *c* were recorded when the incident frequency was below and above resonance, respectively, by one modulation frequency ($\nu_m = 10.8 \text{ MHz}$). Spurious signals at $\pm 10.8 \text{ MHz}$ from the incident frequency and at 1.1 GHz had an amplitude of $\sim -50 \text{ dB}$ with and without sample and have been suppressed.

and high resolution spectral detection would show the same failure of the absorption coefficient approach, whether the absorption coefficient is calculated from population analysis or from consideration of polarisation. On the other hand, either population analysis or polarisation calculations will yield equivalent and correct predictions for usual case in which the time dependence of the total power is detected.

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HERBERT M. PICKETT

Jet Propulsion Laboratory,
California Institute of Technology,
Pasadena, California 91103

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Absorption of hydrogen in 'ReO₃'

RHENIUM TRIOXIDE, ReO₃, has been thought to be a chemically stable compound in air at room temperature¹. The electron diffraction and microscopy measurements reported here reveal, however, that the crystal easily absorbs hydrogen and that this is closely related to its hygroscopic nature. The crystal structure of a new phase, H_xReO₃ ($x < 0.1$), is estimated from the calculation of the potential field.

ReO₃ crystals, of a dark purplish-red colour and having a cubic shape with edge of about 0.5 mm, were prepared by a chemical transport method², and some cubes were crushed in CCl₄. The conventional powder X-ray diffractometer peaks were identified as ReO₃ ($a = 3.75 \text{ \AA}$). On the electron diffraction patterns, however, the crystal fragments gave some weak extra spots besides the fundamental spots of ReO₃. Figure 1a shows a diffraction pattern taken with an incident beam parallel to the [100] direction of ReO₃. The streaks were found to elongate in the the $\langle 001 \rangle$ direction.

The crystal was tilted in the electron microscope to examine the distribution of the diffraction spots and streaks in reciprocal space. All the spots could be indexed on a cubic cell with lattice constant $2a$; extinction occurs for $h + k + l$ odd. The streaks run only along the $\langle 001 \rangle$ directions.

It was first considered that the 'superlattice' was formed by the strain imposed during crushing the crystal. To check this a fine crystal of cubic shape with edge of about $3,000 \text{ \AA}$ was prepared by reducing Re₂O₇ and the electron diffraction patterns were taken without crushing. The distribution of the spots and streaks was substantially the same as mentioned above for the crushed large crystal. Clearly then crushing does not affect the structure of the crystal.

When the crystal was left in air at room temperature, the diffraction pattern gradually changed: the streaks became short, while the superlattice spots became strong in intensity. At the same time, the signals from hydrogens became clear in a NMR measurement³. Figure 1b shows the diffraction pattern from a fragment left in air for about one year. The streaks are no longer observed. The extinction of the spots is the same as before. However, it is difficult to measure quantitatively the amount of hydrogen, since it is still very small.

When the crystals are heated at temperatures between 220 and 230 °C, any signal of hydrogen in NMR becomes inappreciable³. The intensity of the superlattice spots decreases with the increase of heating time but still remains appreciably after heating for 2 months.

On boiling the small crystals in water large amounts of hydrogens are absorbed and an orthorhombic rhenium hydrogen bronze, H_xReO₃, is formed⁴. The crystal gives diffraction patterns slightly different from those above. An example is shown in Fig. 1c, where $x = 0.38$. The lattice parameters are $a = 7.54$, $b = 7.49$ and $c = 7.41 \text{ \AA}$. There is no extinction for the reflections.

When the electron beam is focused on the specimen, the weak spots at the midpoints of the two adjoining strong ones disappear and, at the same time, the symmetry of the reflections becomes cubic. After the transition the array of the spots as well as streaks is similar to that of Fig. 1a. From comparisons with the previous experiment⁴ the transition is seen to be due to the heating by the electron irradiation. Once this transition is complete, no appreciable change follows on further heating even with a more intense electron beam.

These observations indicate that hydrogen is absorbed in ReO₃ in air at room temperature to form a new phase, a cubic rhenium hydrogen bronze, which is also denoted as H_xReO₃, where x is very small, probably less than 0.1. In the present study any 'ReO₃' crystals easily change to this phase in air or even in a desiccator with silica gel. The absorption of hydrogen was suppressed when the crystal was kept in vacuum.

The hydrogen is considered to exist as H⁺ in the crystal. Because the amount is very small, the hydrogen ions must be localised in the potential valley of the ReO₃ subcell, which is formed along the periphery around an oxygen in the (200) plane (Fig. 2). The radius of the circle is almost equal to the conventional bond length of O-H ($0.94 \text{ \AA}$). Protons presumably move around the central oxygen along the valley, in accordance with the NMR measurement³. There are shallow potential minima in the valley and the probability, with which the protons remain, might be slightly higher at these sites.

When the oxygens slightly displace in the $\langle 011 \rangle$ direction, the potential minima become deeper and shift along the valley, as

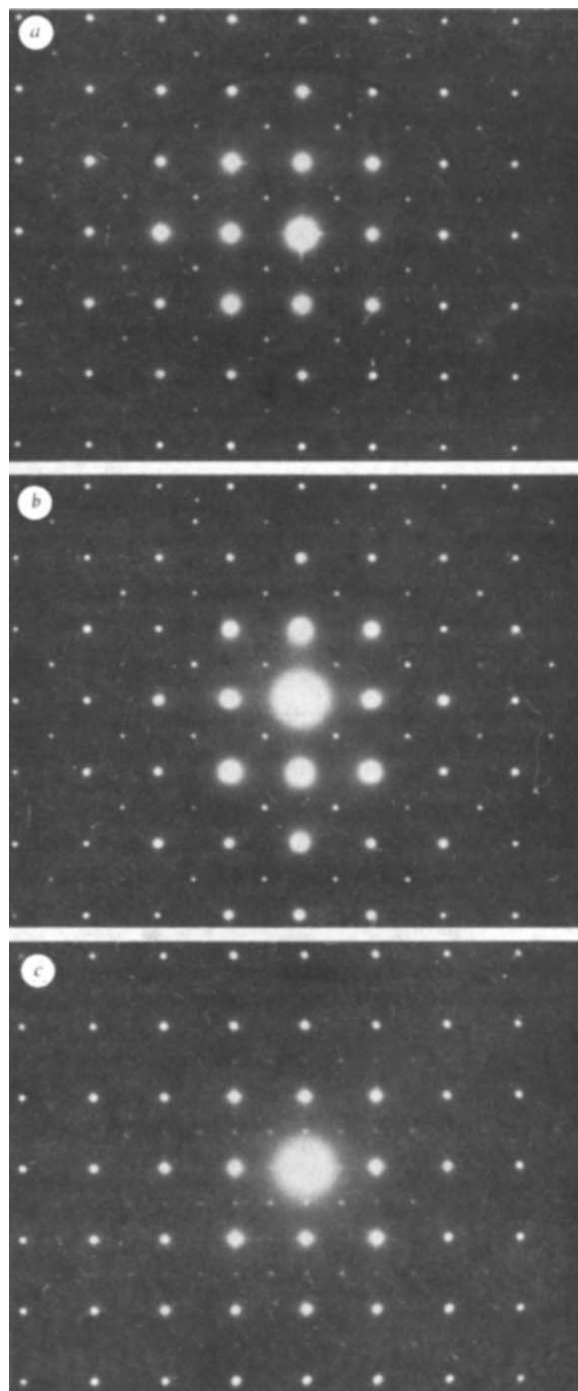


Fig. 1 Electron diffraction patterns from H_xReO₃. *a* and *b* were taken from the crystals left in air for short and long time, respectively ($x < 0.1$); both show a cubic symmetry. *c* was taken from the crystal boiled in water ($x = 0.38$); the symmetry is orthorhombic.

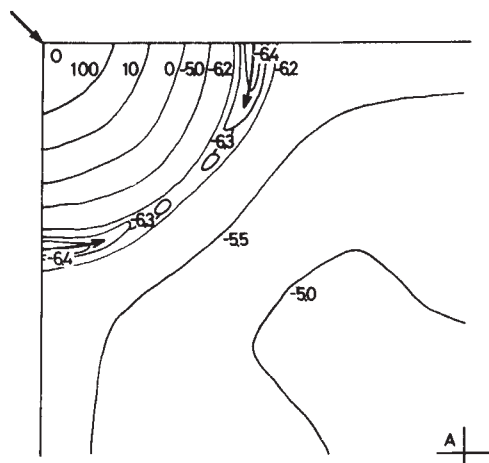


Fig. 2 A potential map for a ReO_3 subcell. One quarter of the (200) plane is shown. A is the cubo-octahedral site and O is occupied by an oxygen. Figure indicates the equipotential level in volts. When the oxygen displaces in the [011] direction, the potential minima becoming deeper shift as indicated by arrows.

indicated by arrows in Fig. 2—the distance O–H remains constant. A similar oxygen displacement is observed in $\text{D}_{0.53}\text{WO}_3$ (cubic, $a = 7.56 \text{ \AA}$, space group $\text{Im}\bar{3}$), whose structure has been determined by neutron diffraction⁵. There is also a similarity not only in the lattice parameter but also in the extinction between the two crystals. It may then be reasonable to assume that the present crystal has almost the same structure as that of $\text{D}_{0.53}\text{WO}_3$, with Re ions occupying the normal positions. There are four possible ways of oxygen displacements in a ReO_3 subcell. Each of them appears in every second subcell in the three {001} directions.

A high-resolution crystal structure image in Fig. 3 was taken by a 1 MeV electron microscope with the incident beam parallel to the [100] direction. The corresponding diffraction pattern was as shown in Fig. 1a (the objective aperture used is indicated by a circle). The contrast of the images taken from very thin parts of crystal at about 700 $\text{\AA}$ underfocus is known to reflect approximately the projection of the structure⁶; the dark dots show the sites of Re ions. The separation of two adjoining dots is about 3.8 $\text{\AA}$, corresponding to the {001} lattice spacing of ReO_3 . The array of Re ions seems perfect, that is, there are no crystallo-

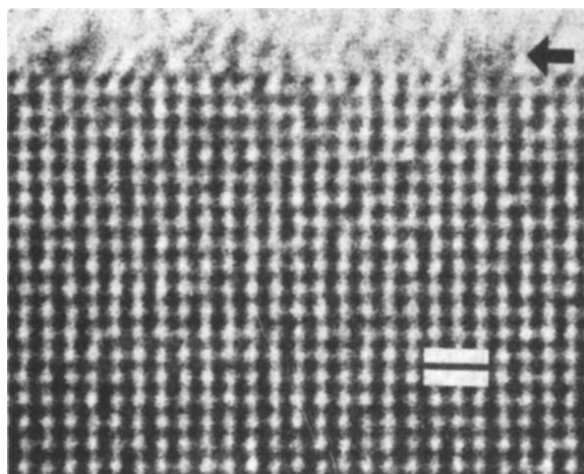
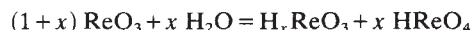


Fig. 3 1 MeV high-resolution electron microscope image of H_xReO_3 ($x < 0.1$). The scale corresponds to 10 $\text{\AA}$. At the crystal surface a thin amorphous-like layer, marked by an arrow, exists below the carbon contamination film formed during observation.

graphic shears. The optical transform of the image gives the reflections only from the ReO_3 subcells.

The diffraction streaks must be due to the disordering of the displacement of oxygens, whose position cannot be clearly read out in Fig. 3. The observation that they are exactly normal to the {001} planes suggests that the stacking faults occur parallel to the planes. When the faults are eliminated with the increase of hydrogen content, the streaks become short and finally disappear.

' ReO_3 ' is strongly hygroscopic. This may be related to the reaction⁴ at the surface of crystal,



The formation of H_xReO_3 means the absorption of hydrogen in the crystal. HReO_4 is normally a liquid at room temperature. At the initial stage of the reaction at the crystal surface, however, it may form a thin solid layer and correspond to the amorphous-like layer thinner than 10 $\text{\AA}$, found in Fig. 3. Such layers have never been observed in any oxide except ReO_3 .

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S. HORIUCHI
N. KIMIZUKA
A. YAMAMOTO

National Institute for Researches in Inorganic Materials,
Sakura-mura, Niihari-gun, Ibaraki, Japan 300-31

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The lowest δD value found in a hydrous silicate, pectolite

MEASUREMENTS of the δD values of waters extracted from specimens of pectolite, collected in Japan and the US, are reported here. The low values (about –400‰) are considered to be a special characteristic of the pectolite crystal structure.

The lowest δD value for meteoritic precipitation on Earth is about –460‰ for the Antarctic ice¹, although δD values of waters extracted from terrestrial minerals and rocks lighter than –180‰ have not been reported previously

$$\left(\delta D\text{‰} = \left[\frac{D/H_{\text{sample}} - D/H_{\text{SMOW}}}{D/H_{\text{SMOW}}} \right] \times 1,000 \right).$$

δD values of hydrogen and water extracted from lunar soils and rocks are as low as –450 to –970‰ (ref. 2). The lowest lunar D/H ratio is almost two orders of magnitude lower than terrestrial values, and the production of hydrogen with such low δD values has been attributed to proton implantation from the solar wind.

According to experimental work on D/H fractionation factor between minerals and water³, δD values of terrestrial minerals are controlled by δD values of ambient waters equilibrated with the minerals, temperature of equilibration and chemical compositions, especially the ferrous–magnesium ratio. Examples of terrestrial minerals showing these controls have been already presented^{4,5}.

We have recently measured δD values of waters extracted from five specimens of the mineral pectolite ($\text{HNaCa}_2\text{Si}_3\text{O}_9$)

Table 1 Water content and δD of pectolites

Locality no.	H ₂ O (wt%)	δD (‰)
1	2.71	-380
2	2.84	-400
3	3.06	-314
4	2.54	-332
5	3.02	-420

- 1, Iwaki-jima, Hiroshima Pref., Japan (syenite)
 2, Horokanai, Hokkaido, Japan (serpentine)
 3, Mitsuishi, Hokkaido, Japan (serpentine)
 4, Suga-shima, Mie Pref., Japan (serpentine)
 5, Paterson, New Jersey, US (serpentine)

from several terrestrial localities. The values obtained (Table I) are surprisingly low, that is, around -400‰. The pectolite from Iwaki-jima occurs as a rock-forming mineral in a syenite, which is a metasomatic facies of a Cretaceous granite in the Chugoku district. All the other pectolite specimens were taken from veins in serpentine bodies. The δD values heavier than -400‰ seem to be mainly due to contamination of the analysed pectolite with other hydrous minerals. For example, pectolites from Suga-shima (no. 4) and Mitsuishi (no. 3) are closely associated with zeolite, and it was difficult to separate the mineral in a pure state, though more than 90% purification was done. Purity of the others was more than 98%.

We next measured δD values of minerals associated with pectolite. The δD values of minerals are known to be affected by the δD value of the ambient water in their crystallisation and in these cases it cannot be assumed that the pectolites are crystallised in the different condition of the associated minerals. Two cases, Iwaki-jima (no. 1) and Suga-shima (no. 4), have been well investigated. Syenite from Iwaki-jima consists of albite (87%, by volume), aegirine (4.0%), sugilite (6.6%) and pectolite (2.3%) with accessory katayamaite, titanite, allanite, andradite, zircon and apatite. Sugilite, a new mineral, corresponds ideally to $(K, Na)((H_2O, Na)_2(Fe^{3+}, Na, Ti, Fe^{2+})_2(Li, Al, Fe^{3+})_3(Si_{12}O_{30}))$ with $Z = 2$ and the space group is $D_{6h}^{2h} - P6/mcc$ (ref. 6). Katayamaite is also a new mineral, calcium-titanium hydrous silicate, which has not yet been fully investigated. Contents and δD values of waters extracted from sugilite, katayamaite, aegirine and albite are 0.67%: -134‰, 1.21%: -143‰, 0.165%: -111‰, and 0.038%: -172‰, respectively. Of course, the waters from aegirine and albite are not structural water, but may be inclusions. Pectolite from Suga-shima occurs as veins with natrolite. The water content and δD value in the latter are 9.50% and -53‰. Equivalent values for serpentine and hornblende from the ultramafic mass of Suga-shima are 12.05~12.98%: -84.8~-98.3‰ and 2.28~3.08%: -55.3~-64.4‰, respectively.

The extraordinarily low δD values of the analysed pectolite specimens are considered not to reflect isotopic equilibration with water, which has an extremely low δD value. Of course, these low values cannot be caused by temperature-falling or Rayleigh fractionation. Instead, the low δD value of pectolite is suggested to be a special characteristic of its crystal structure.

According to Tekeuchi (personal communication), the proton in the pectolite crystal structure forms an O-H-O bridge with apex-oxygen (O_3 and O_4) of SiO_4 tetrahedron and the length of the O-H-O bridge is the shortest of all the minerals with hydrogen bonding (2.473 Å measured by the neutron diffraction). The configuration around hydrogen atom in the crystals critically affects the enrichment or depletion of deuterium in the minerals with hydrogen bonding. Thus, the pectolite will be one of the extreme cases.

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Y. KURODA

Department of Geology,
 Shinshu University,
 Matsumoto 390, Japan

T. SUZUOKI

Marine Department,
 Japan Meteorological Agency,
 Otomachi, Tokyo 100, Japan

S. MATSUO

Department of Chemistry,
 Tokyo Institute of Technology,
 O-okayama, Meguro-ku, Tokyo 152, Japan

Received 29 January; accepted 20 March 1979

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Bracketing dates for two ash falls from Mount Mazama

TEPHRA falls from Mount Mazama^{1,2}, on the site of the present Crater Lake caldera in Oregon, form the most extensive Holocene marker horizon in the Pacific North-west of the US and the adjacent part of Canada. Ash has been found east to Saskatchewan, south to Nevada, north to central British Columbia and on the Pacific Ocean floor³⁻⁶. Although the Mazama ash unit has been radiometrically dated repeatedly in the past 25 years (its age was one of the first determined by Arnold and Libby⁷), problems remain about the nature of the ashfall(s) and the radiometric control for the event(s). For example, the ash shows a degree of petrographic variation that is not attributable only to gravity sorting away from the source. Also the spread of bracketing or included dates for tephra from 6,600 to ~7,000 BP, using charcoal or non-calcareous sediments, is wider than expected for a single Holocene event⁸⁻¹⁰. Taken together, these facts suggest multiple Holocene eruptions of Mount Mazama. Substantial evidence of repeated or multiple eruptions is seen in the two unit sequence of Mazama ash in the C horizons of some soils and in peat bogs in Oregon. The ranges of radiocarbon dates under either the upper or the lower ash layers at several of these sites are significantly different, suggesting that three ash layers may occur¹¹. Near Stillwater, Nevada, at least three ash units can be attributed to Mazama, the lowest of which is petrographically distinct from Mazama ash found elsewhere⁴. We report here the first demonstration that Mount Mazama erupted at least twice within a 200-yr span in the Holocene.

In 1972 R.N.M. extracted two sediment cores to bedrock depth at Bonaparte Meadows (NW 1/4 SE 1/4 S. 20 T38N R30E, Mt Bonaparte Quadrangle elevation 1,020 m), Washington, as part of a Quaternary pollen study¹². Material was collected with a 5-cm Livingstone sampler, and included in each core were two sequential 1-m segments each with a field distinguishable tephra layer.

Samples from each unit were pretreated with hydrogen peroxide to remove organic matter, and with sodium citrate and sodium dithionite to remove iron coatings. Fractions of glass shards were determined by sieving through US Soil Survey sieves. Glass shards were viewed with wide-field microscopy. Phenocrysts were identified and refractive index (RI) was determined with standard light microscope petrographic techniques, while elemental composition of the units was determined with an electron microprobe. These analyses of multiple samples confirmed that both ashes fall within the spectrum of properties attributable to Mazama ash but that they differ in mineralogy and size distribution of glass shards.

Although we had observed laminae in single Mazama ash units from other sites, we had attributed these to secondary deposition and/or deposition into standing water¹³. This is

clearly not the case here as the upper unit is coarser than the lower unit and there is no evidence of fragments or laminae of either unit in the section of the other. Clear separation of the two units by almost 50 cm of included peat (as measured by extracted core length) provides evidence of two distinct ash falls and provides a way of dating these events.

The lower tephra unit (482–490 cm) is made up primarily of silt-size glass shards ($< 50 \mu\text{m}$). Ten per cent of the shards are in the very fine sand (50–100 μm) range. Glass makes up 98% of the sample. Phenocrysts are rare and exclusively plagioclase feldspars. Minor accessory pyroclastic grains include andesite and scoria fragments.

The upper tephra unit (422–434 cm), although it is also primarily glass, has 20% of its sand fraction in the 100–250 μm range. The remainder is mainly very fine sand (50–100 μm). Dacitic glass, brown hornblende, hypersthene, plagioclase feldspar, augite and microlites of magnetite are included. Accessory pyroclastic grains including red and grey andesitic fragments, red scoria and plagioclase from adesitic lithic sources are minor components. Although phenocrysts make up only 10% of the sample they are much more prominent than in the lower unit and these, with the coarser glass fraction, are the major distinguishing features of the units. Glass fractions of the two units have the same RI ($n_g = 1.504$ –1.510, dominantly 1.506–1.508). Elemental analysis of the upper ash unit by electron microprobe gives Ca, K and Fe percentages of 1.23, 2.17 and 1.52, respectively, while the lower unit has the same three elements in percentages of 1.14, 2.12 and 1.54. The proportions of Ca: K: Fe for both units fall within the known limits for Mazama ash¹⁴. A finer particle size unit overlain by a unit with coarser particles matches the stratigraphic arrangement of the ash layers in some of the Oregon bogs and in alluvium in northern Nevada^{4,11}.

Radiocarbon dates obtained from peat above and below each tephra unit allow reasonably tight radiometric dating of the separate ash falls (Table 1). The date for sample TX-2884 may indicate a disconformity in the section, for there is no evidence of barrel-mixing or sampling alteration of the core. Furthermore, we have found a similar hiatus in dates immediately before deposition of the earliest Mazama ash unit at two other sites in the region, indicating possible loss and/or non-deposition of sediment¹⁵.

These dates fall within the range of 6,600–7,000 BP, that most often cited for Mazama ash fall(s), except for the lowest sample, TX-2884, which serves as an older limiting date. These petrographically distinct ash units with their associated dates confirm the earlier field evidence that the stratigraphic marker horizon called Mazama ash was not produced in a single event, but represents at least two separate eruptions. Although the upper three dates are not significantly different, they indicate that these two eruptions most probably occurred within 200 yr of each other.

Confirmation of multiple layers of Mazama ash in the Pacific North-west should bring attention to the areal distribution of each ash fall as well as a prompt search for additional ash units. The nature of possible ash-induced vegetation change during the Hypsithermal interval now deserves reexamination¹⁶. Moreover, the occurrence of additional ash falls sufficiently separated in time to be separated by dated intercalated peat

becomes a tool of considerable importance in the tephrochronology of western North America.

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RICHARD N. MACK
ROSE OKAZAKI

Department of Botany,
Department of Agronomy and Soils,
Washington State University, Pullman 99164

SAM VALASTRO

Radiocarbon Laboratory,
Balcones Research Center,
University of Texas, Austin 78757

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Problems in interpreting tree-ring $\delta^{13}\text{C}$ records

THE stable carbon isotopic ($^{13}\text{C}/^{12}\text{C}$) record of twentieth-century tree rings has been examined^{1–3} for evidence of the effects of the input of isotopically lighter fossil fuel CO_2 ($\delta^{13}\text{C} \sim -25\%$ relative to the primary PDB standard⁴), since the onset of major fossil fuel combustion during the mid-nineteenth century, on the $^{13}\text{C}/^{12}\text{C}$ ratio of atmospheric CO_2 ($\delta^{13}\text{C} \sim -7\%$), which is assimilated by trees by photosynthesis. The decline in $\delta^{13}\text{C}$ up to 1930 observed in several series of tree-ring measurements has exceeded that anticipated from the input of fossil fuel CO_2 to the atmosphere, leading to suggestions of an additional input source of CO_2 ($\delta^{13}\text{C} \sim -25\%$) during the late nineteenth/early twentieth century. Stuiver³ has suggested that a lowering of atmospheric $\delta^{13}\text{C}$ of 0.7‰ from 1860 to 1930 over and above that due to fossil fuel CO_2 can be attributed to a net biospheric CO_2 ($\delta^{13}\text{C} \sim -25\%$) release comparable, in fact, to the total fossil fuel CO_2 flux from 1850 to 1970. If information about the role of the biosphere as a source of or a sink for CO_2 in the recent past can be derived from tree-ring $^{13}\text{C}/^{12}\text{C}$ data it could prove useful in evaluating the response of the whole dynamic carbon cycle to increasing input of fossil fuel CO_2 and thus in predicting potential climatic change through the greenhouse effect of resultant atmospheric CO_2 concentrations. I report here the trend (Fig. 1a) in whole wood $\delta^{13}\text{C}$ from 1883 to 1968 for tree rings of an American elm, grown in a non-forest environment at sea level in Falmouth, Cape Cod, Massachusetts (41°34'N, 70°38'W) on the northeastern coast of the US. Examination of the $\delta^{13}\text{C}$ trends in the light of various potential influences demonstrates the difficulty of attributing fluctuations in $^{13}\text{C}/^{12}\text{C}$ ratios to a unique cause and suggests that comparison of pre-1850 ratios with temperature records could aid resolution of perturbatory parameters in the twentieth century.

There is a decrease in $\delta^{13}\text{C}$ of 0.79‰ for the elm between 1883 and 1933 which closely agrees with the magnitude of the

Table 1 Radiocarbon dates and depth of Mazama tephra at Bonaparte Meadows

Depth (cm)	Uncorrected radiocarbon date (yr BP)	Laboratory No.
415–421	6810 ± 190	TX-2882
422–434	Upper unit	
435–439	6870 ± 110	TX-2881
468–473	6930 ± 110	TX-2883
482–490	Lower unit	
492–497	8300 ± 80	TX-2884

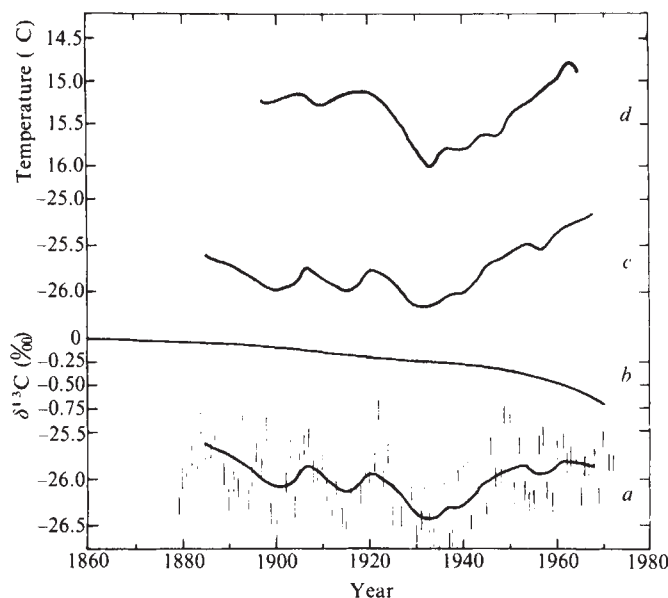


Fig. 1 *a*, Ten-year running mean of $\delta^{13}\text{C}$ in annual tree rings of American elm. (Individual rings containing annual growth wood and sampled radially from tree cross-section were analysed. The individual $\delta^{13}\text{C}$ measurements are plotted with an associated analytical error of $\pm 0.1\%$ ($\pm 2\sigma$).)

$$\delta^{13}\text{C}_{\text{sample}} (\text{‰}) = \frac{R_{\text{sample}} - R_{\text{PDB}}}{R_{\text{PDB}}} \times 1,000$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$. *b*, Estimated decline in atmospheric $\delta^{13}\text{C}$ due to input of fossil fuel CO_2 (after Stuiver³ and Oeschger *et al.*⁵). *c*, Ten-year running mean of $\delta^{13}\text{C}$ in American elm after correction for fossil fuel CO_2 input to the atmosphere (curve *a*—curve *b*). *d*, Ten-year running mean of mean annual summer (April to September) temperatures for Nantucket Island.

decline reported for the same period by Stuiver (Fig. 2 in ref. 3) for the cellulose fraction of Douglas fir tree rings from the west coast of the US. Stuiver estimated the contribution of fossil fuel CO_2 to the decline in $\delta^{13}\text{C}$ of atmospheric CO_2 from the model of Oeschger *et al.*⁵ designed to account for the measured reduction to 1950, by tree-ring analysis, of the ${}^{14}\text{C}/{}^{12}\text{C}$ ratio in atmospheric CO_2 by the input of non- ${}^{14}\text{C}$ containing fossil fuel CO_2 and subsequent carbon isotopic exchange and CO_2 transfer between reservoirs of the carbon cycle. Figure 1*b* shows the anticipated decline in atmospheric $\delta^{13}\text{C}$ from 1860 to 1970 based on Stuiver's use of Oeschger *et al.*'s model, taking $\delta^{13}\text{C}$ of fossil fuel CO_2 as -18% relative to $\delta^{13}\text{C}$ of atmospheric CO_2 , and extrapolating on the basis of fossil fuel CO_2 input data for the period 1950–70 when the atmospheric ${}^{14}\text{C}/{}^{12}\text{C}$ ratio was significantly elevated by artificial ${}^{14}\text{C}$ production from nuclear weapons' testing. The calculated decline due to fossil fuel CO_2 is 0.25% by 1933 of which 0.21% applies for the interval 1883–1933. Therefore the reduction in atmospheric $\delta^{13}\text{C}$ after correction for fossil fuel CO_2 addition seems to be 0.58% for 1883–1933 (Fig. 1*c*). This corresponds closely to the 0.55% reduction for 1880–1930 out of the 0.7% decline for 1860–1930, postulated as being due to net biospheric CO_2 release by Stuiver (Fig. 3 in ref. 3) after fossil fuel CO_2 correction applied to a combination of his own and other selected $\delta^{13}\text{C}$ profiles.

The clearing of forests and the ploughing of grassland releasing additional CO_2 to the atmosphere, the 'pioneer agriculture explosion', has been invoked to account for the remarkable 0.9% decline in $\delta^{13}\text{C}$ from 1880–90 in Bristlecone pine cellulose⁶, and the $\delta^{13}\text{C}$ decreases of 1 to 1.5% from 1900 to 1920 in British wood¹. While the decline in $\delta^{13}\text{C}$ of the American elm in this study is spread over several decades to 1933, it also apparently supports the concept of net biospheric CO_2 release but corresponds more closely, in magnitude and trend, to that reported by Stuiver³.

From 1933 to 1968 there is a reversal in trend, measured $\delta^{13}\text{C}$ of the American elm increasing by 0.58% (Fig. 1*a*). While most investigations have found a decline in $\delta^{13}\text{C}$ to around 1930, trends since then have been less uniform although increases in $\delta^{13}\text{C}$ over part or all of the post-1930 period have been found^{1,2,7}. Stuiver³ reported a 0.5% increase in the sapwood (post-1932) portion of the Douglas fir but did not include sapwood $\delta^{13}\text{C}$ data in his $\delta^{13}\text{C}$ curve due to possible changes in chemical composition of the wood. However, he did estimate that only one-tenth at most of this increase could be explained by differences in lignin content due to residual additional lignin in cellulose extracted from heartwood. (No information is available on possible changes in the relative proportion of wood constituents in the American elm in which the heartwood/sapwood transition occurs in 1923.) He did suggest, however, that this increase in $\delta^{13}\text{C}$ and the 1% increase in $\delta^{13}\text{C}$ of a Tasmanian tree⁷ after 1950 could reflect a cessation of net biospheric CO_2 release. Such a phenomenon would be expected to restore a fossil fuel CO_2 corrected $\delta^{13}\text{C}$ curve to $\delta^{13}\text{C}$ values pertaining in the mid-nineteenth century. For the American elm (Fig. 1*c*), the corrected $\delta^{13}\text{C}$ trend increases by 0.96% from 1933 to 1968 and attains a more positive $\delta^{13}\text{C}$ value (by 0.38%) than that of 1883 and, also, than a theoretical value for 1860 were a 0.15% $\delta^{13}\text{C}$ decline to pertain for 1860–80 as in Stuiver's study (Fig. 3 in ref. 3). Whether, beyond a cessation of net biospheric CO_2 release, an increase in biomass is indicated by the size of this $\delta^{13}\text{C}$ increase is unclear; however, it would oppose some opinions on the present state of the terrestrial biomass⁸.

Pearman *et al.*⁷ found a significant correlation between trends in regional summer temperature and $\delta^{13}\text{C}$ of the Tasmanian trees, during both the period of $\delta^{13}\text{C}$ decrease from 1895 and the post-1950 period when measured $\delta^{13}\text{C}$ increased to the 1895 level. Figure 1*d* shows the mean summer (April–September) temperature trend for nearby Nantucket Island, some 50 km south-east of the growth site of the American elm. The temperature decreases by 1.3 °C from 1933 to the 1960s and matches the trend to more positive $\delta^{13}\text{C}$ (Fig. 1*c*), suggesting a temperature dependent fractionation process (nominally -0.7% per °C) during photosynthesis. Note that a decline in $\delta^{13}\text{C}$ from 1919 to 1933 of 0.46% (Fig. 1*c*) is accompanied by an increase in temperature of 0.9 °C. This apparent relationship between $\delta^{13}\text{C}$ and temperature may be fortuitous and a definitive assessment must await comparison of $\delta^{13}\text{C}$ and temperature records from the pre-industrial era.

Grinsted *et al.*⁹ have recently reported that $\delta^{13}\text{C}$ variations for bristlecone pine cellulose from North America over the past 1,000 years are similar to English high summer temperature variations for the same time period. The apparent relationship is much larger in magnitude and opposite in direction to that previously reported for New Zealand *Pinus radiata* cellulose¹⁰ ($+0.2\%$ per °C) and the Tasmanian trees ($+0.24$ to $+0.48\%$ per °C) of Pearman *et al.*⁷ during the twentieth century. Despite the geographical incongruity of Grinsted *et al.*'s $\delta^{13}\text{C}$ and temperature data and their need to consider other possible influences (such as precipitation and humidity), Grinsted *et al.*'s major finding resembles quite remarkably that indicated by the 'fossil-fuel CO_2 corrected' $\delta^{13}\text{C}$ –temperature correlation for the elm post-1933.

Note that should the elm have been subject to an enhanced 'regional' fossil fuel CO_2 influence relative, for example, to the west coast Douglas fir, due to the eastwards movement of continental air from the industrialised and well-populated eastern states of the US, the effect would have been to render $\delta^{13}\text{C}$ of the elm relatively more negative. Both the agreement in $\delta^{13}\text{C}$ decline between the elm and the Douglas fir to the early 1930s and the reversal of $\delta^{13}\text{C}$ trend for the elm post-1933 suggest that any such additional 'regional' fossil fuel CO_2 influence on the elm has been insignificant relative to the major perturbatory parameters summarised below.

The striking similarity between temperature and $\delta^{13}\text{C}$ trends, especially after 1933, must cast some doubt on the interpretation of twentieth century $\delta^{13}\text{C}$ trends via consideration

solely of fossil fuel CO₂ input and of the role of the biosphere, although arguments for $\delta^{13}\text{C}$ decline to 1930 based on net biospheric CO₂ release as a consequence of agricultural activity since 1860 are equally persuasive. It seems clear that a resolution of the various parameters which may potentially affect atmospheric $\delta^{13}\text{C}$ will be forthcoming only after detailed study of tree-ring $^{13}\text{C}/^{12}\text{C}$ ratios before the onset of man's major industrial and agricultural activities on a global scale and for a geographical area where lengthy direct temperature records are available¹¹. Also, despite fair agreement in twentieth-century $\delta^{13}\text{C}$ trends between different studies based on whole wood and cellulose fractions, notably between the American elm here and the Douglas fir of Stuiver³, investigation of possibly significant $\delta^{13}\text{C}$ changes in some tree species due to variations in the chemical composition of wood could be valuable. These studies could be of considerable use both in historical climatology and for an understanding of the effects of the impingement of human activities on the carbon cycle more recently.

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JOHN G. FARMER*

Department of Chemistry,
Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543

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* Present address: Department of Forensic Medicine, University of Glasgow, Glasgow, UK.

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Metal co-tolerances in the grass *Deschampsia cespitosa*

METAL tolerance in plants growing on metal-contaminated soils around mines^{1–3}, smelters⁴ and refineries⁵ have been reported. While metal tolerances in many dicotyledonous species, such as *Becium homblei*⁶, *Mimulus guttatus*⁷ and *Silene cucubalus*⁸, have been found, the emphasis has largely been placed on the study of populations of a limited number of grass species, especially *Agrostis tenuis*^{1,2,9–11}, *Agrostis stolonifera*^{5,12}, *Festuca ovina*³ and *Anthoxanthum odoratum*^{13,14}. (Adaptive population responses to a number of elements high in soils were recorded for lead, zinc, copper and nickel, reviewed by Antonovics *et al.*¹⁵.) In addition, tolerances to cadmium⁴ and arsenic¹⁶ have been investigated. Jowett¹⁷ and Bradshaw *et al.*¹¹ have postulated that tolerance to more than one element is dependent on the presence of these elements at elevated levels in the soil, that is, tolerance is specific. A few exceptions to this 'rule' have been noted, such as the apparent enhancement of nickel tolerance in a zinc-tolerant population of *Agrostis tenuis* despite the absence of elevated soil nickel¹¹. We present here evidence of the evolution of co-tolerances to copper, nickel and aluminium in a population of *Deschampsia cespitosa* (L.) Beauv. Also, the occurrence of co-tolerances for lead and zinc, which were not elevated in the soils from which the population was obtained. A limited number of cadmium-tolerant clones were also found in both populations.

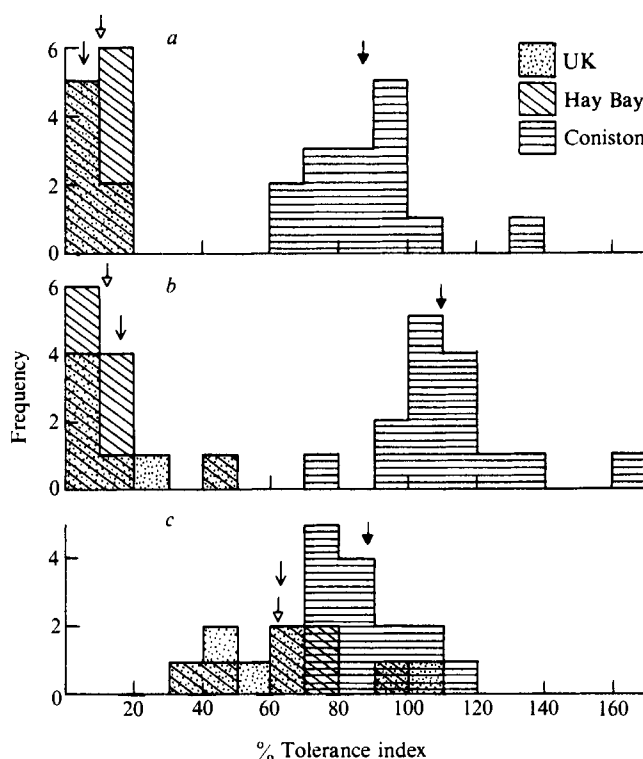


Fig. 1 The distributions of copper (a), nickel (b) and aluminium (c) tolerances for clones of *Deschampsia cespitosa* sampled from around a copper-nickel smelter at Coniston, Ontario, and uncontaminated populations at Hay Bay, Ontario and from Derbyshire, UK. Solution levels were 0.3 p.p.m. Cu, 0.3 p.p.m. Ni and 1.35 p.p.m. Al. ↓, Mean for UK population; ↓, mean for Hay Bay population; ↑, mean for Coniston population.

Deschampsia cespitosa has invaded the metal-contaminated barren lands around the Coniston nickel-copper smelter at Sudbury since its closure in 1972. The Sudbury area has been subjected to intense SO₂ fumigations, as well as to soil contamination by metal fallout over the 60–70 yr since the major smelters opened^{18,19}. Acid precipitation has been an added factor²⁰. The resultant increased soil acidity has increased aluminium solubility, as well as maximising the solubility of the elevated nickel and copper contents of the soil¹⁹. The tolerances of a Coniston area population to nickel, copper, aluminium, lead, zinc and cadmium in solution culture have been compared with a population sampled from uncontaminated pastures at Hay Bay on the Bruce Peninsula, located 150 km from Sudbury. A second 'control' was used in some experiments, this being obtained from an uncontaminated pasture in Derbyshire, UK. The lead, zinc and cadmium levels in the Coniston soils were shown not to be elevated, while the copper, nickel and available aluminium levels were elevated (Table 1).

The populations of *D. cespitosa* at both Coniston and Hay Bay were sampled as single tillers from individually identified clones, whereas the Derbyshire population was raised from seed. Each clone was allowed to grow in a greenhouse soil mix for nine months before experimentation.

The metal tolerance of each clone was determined using the method of Wilkins³, where 20 tillers were allowed to root simultaneously in water culture for 10 d, in the presence and absence of the particular metal in standard environmental conditions. The longest root of each tiller was then measured. The ratio, as given by:

$$100 \times \frac{\text{Mean length of the longest root of 20 tillers grown in the metal amended solution}}{\text{Mean length of the longest root of 20 tillers grown in the control solution}}$$

Table 1 Soil analysis of rooting zone soil from Canadian populations

Site	pH	Total digest (hydrofluoric-perchloric) Metals ($\mu\text{g g}^{-1}$ air-dried soil)						
		Ni	Cu	Pb	Cd	Zn	Al	Ca
Coniston	3.5–3.9	423	359	25	nd	77	—	—
Hay Bay	6.8–7.2	22	19	72	nd	66	—	—
Water extractable metals								
Coniston		24	8.5	0.2	0.04	3.4	2.8	45
Hay Bay		0.1	0.2	0.2	nd	0.2	0.2	483

Results represent the means of 3 samples.
nd, below detection limit.

is known as the index of tolerance and was calculated for each clone (Figs 1, 2). The culture solution used was 0.5 g dm^{-3} calcium nitrate, at pH 5.5 and was changed every 3 d. The testing for aluminium tolerance required careful pH control. To this end 0.3 g dm^{-3} calcium chloride was used, and adjusted to a final pH of 4.5 with HCl. This solution was changed daily to maintain the pH and metal concentration. The metals were added as chloride except for lead, which was added as the nitrate. The metal concentrations used for testing were 0.3 p.p.m. for nickel and copper, 1.35 p.p.m. for aluminium, 2.0 p.p.m. for zinc and lead and 1.0 p.p.m. for cadmium. These concentrations represent those that produced the largest significant differences in relative root growth between Coniston and control representative clones in preliminary experiments, in which the responses to a range of concentrations were tested.

As the distributions of the tolerance indices for most metals were highly skewed and large differences in root growth were recorded, a log index of tolerance was thought more suitable for statistical analysis of the data. The raw data were transformed using a formula of Wilkins:

$$1 + \log \frac{\text{mean length of the longest root of 20 tillers grown in control solution}}{\text{mean length of the longest root of 20 tillers grown in the metal amended solution}}$$

This did not eliminate skewness altogether in the nickel and copper data but there was more confidence that comparisons were being made on similar patterns of distributions. As no assumptions were made as to the normality of the data, the non-parametric Mann and Whitney *U* statistic (for $2n = 9-20$) for a two-tailed test and the Kruskal-Wallis one-way analysis of variance by ranks (*H*) was used.

Population differences (*H* statistic) in copper and nickel tolerances were found to be significant at the 0.1% level, while differences in aluminium tolerance occurred at the 5.0% level of significance (Table 2). The Mann-Whitney test (*U*) revealed that tolerances of the two control populations were each different to those of the Coniston population at the 0.1%, 0.1% and 5.0% levels for nickel, copper and aluminium respectively. No such differences were found between the two control populations. Copper and nickel tolerances at the test concentration of

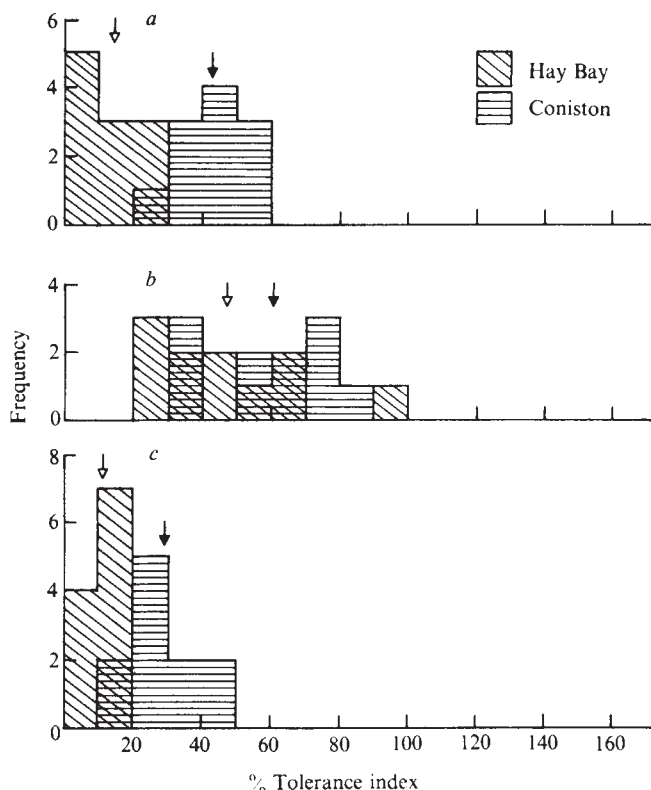


Fig. 2 The distributions of lead (a), cadmium (b) and zinc (c) tolerance indices for clones of *Deschampsia cespitosa* sampled from around a copper-nickel smelter at Coniston, Ontario and from an uncontaminated population at Hay Bay, Ontario. Solution levels used were 2 p.p.m. Pb, 1 p.p.m. Cd and 2 p.p.m. Zn. $\downarrow$, Mean for Hay Bay population; $\downarrow$, mean for Coniston population.

0.3 p.p.m. showed a complete separation in response between those individuals from Coniston and those from control sites (Fig. 1).

In addition, significant differences ($P < 0.001$) were found between the Hay Bay control and the Coniston population in both their lead and zinc tolerance. These differences could not have been anticipated on the basis of the low levels of these two metals in the Coniston soil (Table 1). Lead is actually at a higher total concentration in the Hay Bay soil, though the high pH rendered it largely insoluble. No differences were found between the populations in their cadmium tolerance, although it should be noted that the level of tolerance found here approaches that reported by other workers for plants they describe as cadmium tolerant⁴.

The data suggest that the evolution of tolerance to nickel, copper and aluminium in response to elevated soil levels around Sudbury has coincidentally increased tolerance to lead and zinc in the Coniston population despite the lack of elevated levels of these metals in the soil from which they were obtained. The occurrence of coincidental tolerances resulting from evolution

Table 2 Means and ranges of transformed tolerances to various metals of three populations of *Deschampsia cespitosa*

Metal	Coniston		Hay Bay		Derbyshire		<i>H</i>
	Mean	Range	Mean	Range	Mean	Range	
Ni	0.96	0.79–1.06	1.97†	1.32–2.29	1.95†	0.98–1.44	23.9†
Cu	1.06	0.88–1.17	2.01†	1.72–2.21	2.34†	1.80–2.85	24.9†
Al	1.06	0.96–1.15	1.21*	1.03–1.40	1.23*	1.30–2.37	8.84*
Cd	1.24	1.07–1.50	1.36	1.03–1.62			
Pb	1.37	1.25–1.52	1.90†	1.53–2.36			
Zn	1.55	1.32–1.75	1.98†	1.74–2.24			

Probabilities represented are those determined from the Mann-Whitney *U* statistic for differences between the Coniston population and each of the two control populations. *H*, the Kruskal-Wallis one-way analysis of variance by ranks. Both tests were carried out according to Siegel²¹.

* $P < 0.05$. † $P < 0.001$. Note that low values mean high tolerance.

to specified metal pollutants has had limited support. Co-tolerance to nickel was reported in a zinc tolerant population of *Agrostis tenuis*, despite the lack of nickel in the soil¹¹. Copper-tolerant *Mimulus guttatus* was reported to have slightly elevated tolerances to nickel and zinc⁷. Cadmium-tolerant bacteria and yeasts were found to be resistant to additional metals²². Also, the unicellular green algae *Scenedesmus acutiformis* and *Chlorella fusca* collected from nickel-copper polluted Sudbury lakes showed not only nickel and copper tolerance but also silver tolerance²³.

The present work contravenes the generally accepted view that tolerance is element-specific and evolves as a response to elevations of the appropriate metals in the soil. It suggests the occurrence of at least some common physiological mechanism of metal tolerance, by which the occurrence of a mechanism for dealing with one metal may confer tolerance to one or more other metals which are not at elevated levels in their environment. Investigations of multi-element tolerances in native plant populations not only increases our understanding of evolutionary processes and physiological responses but is integral in the assessment of certain adaptive or preadaptive traits necessary for land reclamation purposes.

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R. M. COX

T. C. HUTCHINSON

Department of Botany and Institute for Environmental Studies,
University of Toronto,
Toronto, Ontario, M5S 1A1, Canada

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Table 1 Positions of eggs in three incubating ostrich nests

Eggs laid by	No. of eggs in centre (being incubated)	No. of (doomed) eggs in outer ring	P
Nest A			
Major hen	9	0	0.020
4 other hens	10	8	
Nest B			
Major hen	13	0	0.011
2 other hens	6	5	
Nest C			
Major hen	9	1	0.029
4 other hens	9	10	

Few studies of the breeding of the large wild ratites have been published^{2,6–10}, and none on the Masai ostrich (*S. c. massai-cus*)^{4,11}. Data were collected in Tsavo West National Park, Kenya, during July–October 1977 and 1978, and (one nest) Serengeti National Park, Tanzania, in June–July 1973.

Nests were found mainly by observing ostriches from 0.3 to 2.0 km away, for birds near their nests are exceedingly wary². To provide useful data, nests had first to survive the 3 weeks until incubation (43% of 37 known nests survived predators this long); second, to contain by then enough eggs (>20) for some to be pushed out (75% of the 16 incubating nests did); and third to have been discovered by me early enough to determine which eggs were laid by the major hen (only three of these 12 nests).

Every egg was numbered (by small scratches in the shell), weighed, measured, photographed and surface characteristics described. To minimise disturbance, nests were visited only once every 1–3 days. I determined which were the major hen's eggs by direct observations of birds laying, time-lapse photography, knowing that ostriches lay at 2-day intervals¹ and consistency within individuals in egg characteristics (unpublished data).

Time-lapse photography at three nests showed 14 instances of major hens pushing out eggs around the start of incubation, but no case of any other bird doing so.

Table 1 shows the arrangement of eggs in the three usable nests: the minor hens' eggs were selectively evicted. Two of the three major hens discriminated perfectly. I do not know how or why the major hen in nest C had her own first laid egg (of 1,341 g) also pushed out. Minor hens who laid more than two eggs had some inside and some pushed out. Thus the major hen was not recognising a particular individual's eggs and evicting them, but recognising her own and retaining them.

How she recognises her own eggs is unclear, but there are several possible methods. Table 2 shows that in these nests discrimination by egg size relative to her own (known) egg size would be feasible, but that this is probably not the basis of discrimination among minor hens' eggs. Details of egg shape and surface texture also provide possible but difficult clues. She does not use the age of the egg, nor the place in the nest that it was laid (unpublished data). Discrimination by smell is improbable.

The causes, costs and benefits of the flexible major and minor strategies are being analysed⁴. Extra eggs buffer the major hen's eggs against predation (unpublished data). Clearly, an egg laid in

Ostriches recognise their own eggs and discard others

WILD ostriches (*Struthio camelus* L.) generally use communal nests^{1–4}. In East Africa, 2–7 hens lay up to 13 eggs each in the same shallow scrape in the ground. Only one, the 'major' hen², guards and later incubates the nest, helped by the male. Although a complete nest may contain up to 30–40 of the white 1.5-kg eggs^{4,5}, an ostrich hen can incubate only about 20 (unpublished data). The surplus are pushed out to 1–2 m away where they are not incubated, and perish. I report here that the major hen avoids pushing out her own eggs.

Table 2 Mean weights (and standard deviations) of ostrich eggs (g)

	Major hen's eggs	Minor hens' eggs		No. of minor hens' eggs within size range of major hen's eggs
		Retained eggs	Pushed out eggs	
Nest A	1,378 (±40)	1,556 (±70)	1,596 (±58)	1
Nest B	1,748 (±61)	1,487 (±48)	1,476 (±40)	0
Nest C	1,361 (±54)	1,628 (±81)	1,641 (±116)	0

Numbers of eggs and of birds are shown in Table 1.

a large nest has approximately a twofold survival advantage if it is in its mother's nest rather than in another female's nest. In none of the few other communally-laying species^{8,12} have birds been shown to be able to recognise their own eggs. The ability opens up new complexities in the evolution of 'cooperative' breeding systems.

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BRIAN C. R. BERTRAM

The Research Centre,
King's College,
Cambridge, UK

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New look at the origin of birds and crocodiles

DURING the period 1926-73 most ornithologists and vertebrate palaeontologists supported Heilmann's theory of avian origins. Heilmann¹ argued that all dinosaurs and pterosaurs were too specialised to have been ancestral to birds. Instead he chose to derive birds directly from a primitive group of Triassic archosaurs, the Pseudosuchia. Heilmann's theory has recently been challenged by Walker²⁻⁴, who has suggested that birds evolved from an early crocodilian, and by Ostrom⁵⁻¹⁰, who argued that birds descended from theropod dinosaurs. We recently began a study of the otic region in archosaurs and birds, in search of advanced, homologous features which would give definitive support to one theory or the other. The results of this investigation, discussed below, strongly support a hypothesis of a common pseudosuchian origin for birds and crocodiles, independent of dinosaurs.

The otic region of the living rhynchocephalian, *Sphenodon*, is usually assumed to represent the primitive amniote condition¹¹, although the middle ear is somewhat specialised. In all tetrapods the inner ear is composed of two distinct fluid-filled compartments, an endolymphatic compartment which contains the sensory apparatus for hearing and orientation, and an investing perilymphatic compartment (Fig. 1b). In the vestibular region the endolymphatic channel is drawn out into a short lobe, usually termed the lagena, which contains the sensory hair cells of the basilar membrane. Sound vibrations from the stapes are transmitted to the sensory membrane by a perilymphatic duct which is wrapped around the lagena. Compressional waves carried by the duct must be dissipated outside the otic capsule. In primitive reptiles, the perilymphatic duct leaves the otic capsule in the internal wall of the braincase, passes along a groove in the braincase wall and is exposed to suprapharyngeal tissues after passing through the fissura metotica (vagus foramen). In addition to transmitting the perilymphatic duct, the metotic fissure of *Sphenodon* and primitive reptiles provides an exit for the ninth to eleventh cranial nerves. In birds and crocodiles, the perilymphatic duct leaves the otic capsule lateral to its primitive position

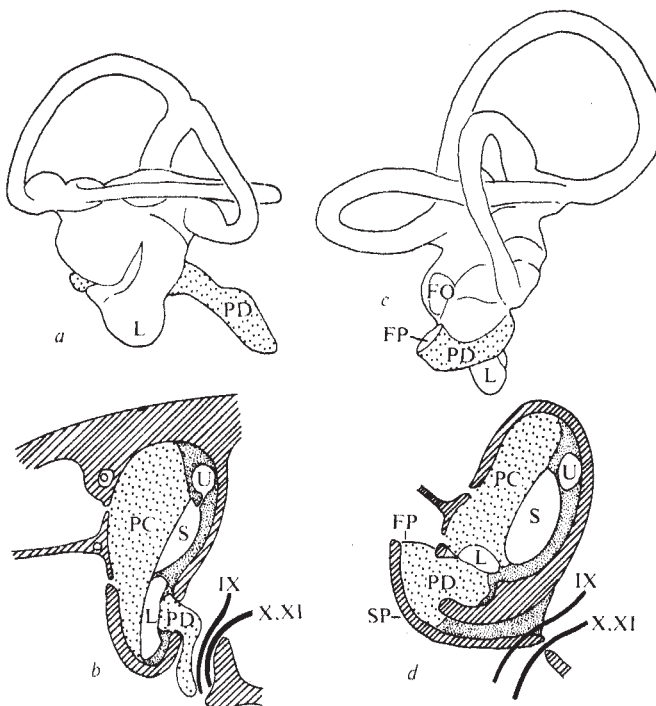


Fig. 1 Inner ear of the rhynchocephalian, *Sphenodon* (left), compared with that of birds and crocodiles (right). The perilymphatic compartment or its terminal portion is coarsely stippled. a, b Left ear of *Sphenodon* in lateral aspect (a) and transverse section (b), after Retzius²³, de Burlet²⁴ and Baird¹¹; c, the crow, *Corvus*, in lateral aspect, after Gray²⁵; d, a crocodylid in transverse section, after Baird¹¹. L, S, U, endolymphatic compartment; PD, perilymphatic duct; PC, perilymphatic cistern; FP, fenestra pseudorotunda ('round window'); FO, fenestra ovalis; SP, subcapsular process; IX-XI, cranial nerves.

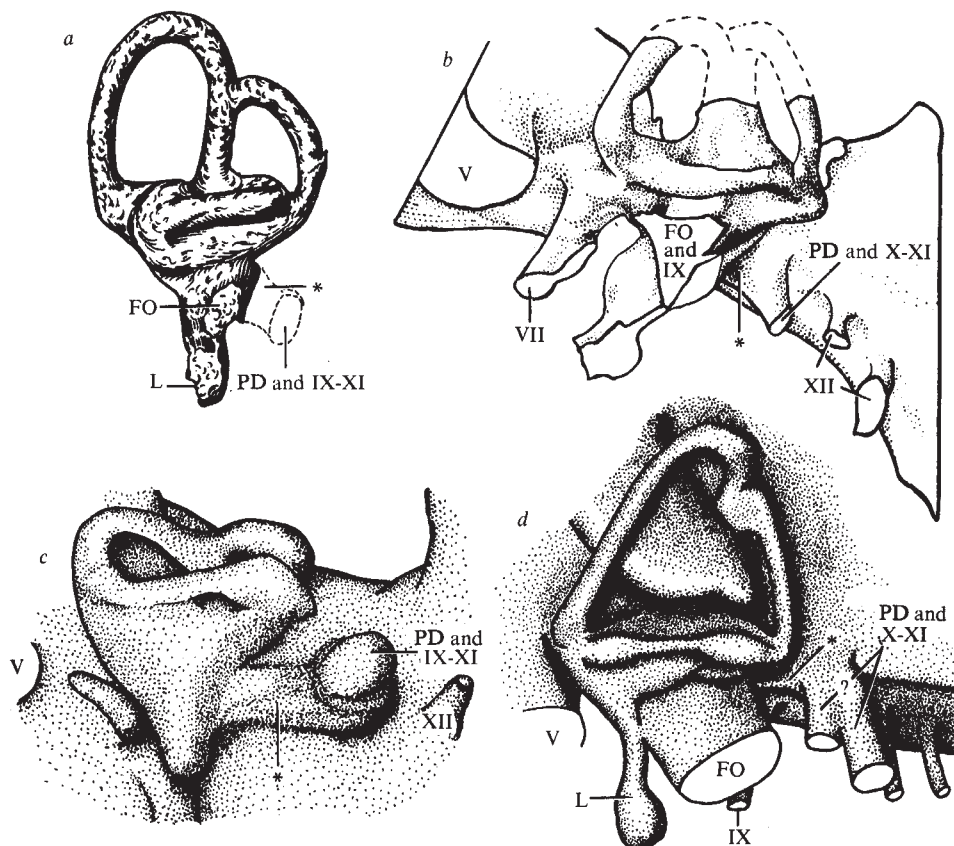


Fig. 2 Casts of the inner ear and cranial cavity of saurischian and ornithischian dinosaurs. * Indicates the cast of the groove in the braincase wall for the perilymphatic duct. *a*, The sauropod, *Brachiosaurus*, after Janensch²⁶ and Lapparent and Lavocat²⁷; *b*, the hadrosaur, *Lophorothon*, after Langston²⁸; *c*, *Ankylosaurus* (AMNH/American Museum 5214, right ear reversed to appear left); *d*, the theropod, *Allosaurus* (UUV/Univ. Utah 294, the cranioquadrate region is visually sectioned to allow view from this angle). V–XII, cranial nerves; other abbreviations as in Fig. 1.

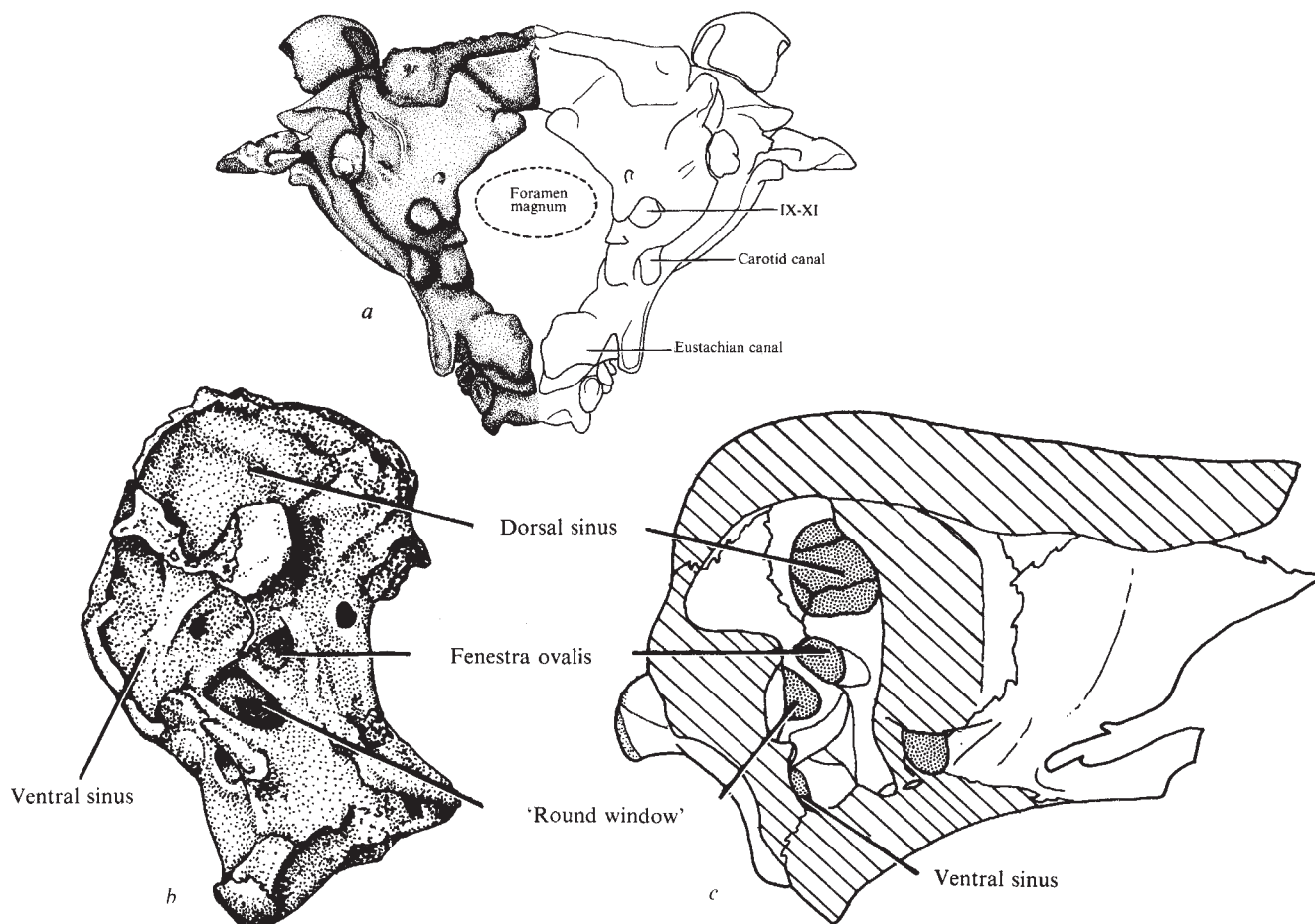


Fig. 3 Pneumatic spaces in the ear region of crocodiles and birds. *a*, Posterior view of a cast of the pneumatic spaces in the periotic bones of Recent *Alligator*, with associated structures indicated; *b*, lateral view of the middle ear region of *Hesperornis* (FMNH/Field Museum PA 219); *c*, ear region of a crocodylid in lateral aspect.

Hesperornis (Fig. 3b) and *Sphenosuchus*^{2,3} had perilymphatic ducts which issued laterally from the lagenar recess into a fenestra pseudorotunda. The fenestra probably arose from a canal for the ninth cranial nerve which was 'captured' by a lateral shift of the perilymphatic duct. This eliminated the necessity for an unossified vestibular region in the braincase wall (as the duct no longer used this space to leave the otic capsule) and allowed the complete internal ossification of the adult otic capsule, a feature already present in *Hesperornis* and the Triassic crocodile, *Notochampsia* (BMNH R 8503). Although other living vertebrates (mammals and lizards) also have round windows, only in birds and crocodiles is this feature formed, embryologically, by a subcapsular process. This process is a cranial rib, ossified in the adult, which divides the metotic fissure and forms the floor and posterior wall of the fenestra¹². In the adult it also forms a laterally directed flange, fused to the combined exoccipital/opisthotic bone at the back of the middle ear cavity, and supporting the secondary tympanic membrane (Fig. 1d). Such similarity in the developmental process would be unlikely if the fenestra pseudorotunda of birds and crocodiles was independently derived.

An additional feature shared by birds and crocodilians is a system of pneumatic spaces in the bones surrounding the middle ear (Fig. 3). An upper pneumatic chamber joins the middle ear cavity beneath the lateral shelf of the squamosal bone at its junction with the prootic and exoccipital/opisthotic bones. Lying just above the principal braincase articulation with the quadrate, this cavity extends medially and dorsally into the supraoccipital and parietal bones. A second chamber, below and behind the quadrate articulation, extends into the base of the paroccipital process. These periotic sinuses also occur in Mesozoic birds (*Hesperornis*) and Triassic crocodilians (*Notochampsia*), but are absent in theropod dinosaurs and other sauropsid reptiles. Functionally, periotic pneumatic cavities represent an enlargement of the air cushion which lies medial to the tympanic membrane. They are thus analogous to the enlarged auditory bullae or pneumatic petrosal bones of mammals. The dampening effect of the air cushion is such that a large middle ear cavity is better suited for low frequency sound, and a small cavity is better suited for high frequency^{20,21}.

These advanced features in the ear region support a hypothesis of common ancestry for crocodiles and birds, independent of both saurischian and ornithischian dinosaurs. A hypothetical common ancestor having a fenestra pseudorotunda and periotic pneumatization, but also having the primitive features retained by either birds or crocodiles (for example, cranial kinesis and 'semi-improved'²² locomotion), would currently be classified within the paraphyletic grade, Pseudosuchia. A theory of a pseudosuchian origin for birds is, at the very least, a reasonable alternative to the dinosaur model proposed by Ostrom.

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K. N. WHETSTONE*
L. D. MARTIN

Museum of Natural History
and Department of Systematics and Ecology,
University of Kansas, Lawrence, Kansas 66045

Received 11 January; accepted 6 March 1979.

* Present address: Dept of Palaeontology, British Museum of Natural History, Cromwell Road, London SW7 UK.

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Transplantation of a circadian pacemaker in *Drosophila*

A MAJOR question in the physiology of activity rhythms is the nature of the coupling between the pacemaker and the motor system. There is good evidence for humoral coupling in several species. In the sparrow, pineal organs from donor birds maintained in a light-dark cycle different from that of the host were transplanted into the anterior chamber of the eye of recipient birds made arrhythmic by pinealectomy. In this case the activity rhythm of the host was restored and its phase was determined by a diffusible humoral factor from the implanted pineal¹. In the mollusc *Aplysia* humoral coupling between the circadian pacemaker and the output cells is thought to control locomotor activity, although electrical coupling has not been ruled out². In insects, however, experiments involving cockroaches and silkworms indicate that an intact electrical connection between the brain and thoracic ganglia is required for rhythmic activity. The pacemaker controlling the locomotor activity rhythm in the cockroach appears to reside in the optic lobes, and disruption of the electrical pathway between the optic lobes and thoracic ganglia results in arrhythmicity^{3–6}. Initial transplant experiments with this insect indicated that activity could be hormonally controlled by the sub-oesophageal ganglion⁷. While these results have not been repeatable by other laboratories^{8–10}, evidence from parabiosis experiments does suggest some humoral influence on the activity rhythm¹¹. Surgical experiments using giant silkworms have also demonstrated the need for an intact neural connection between brain and thorax for expression of the flight activity rhythm¹². Thus, until now, there has been no unambiguous evidence for humoral control of activity rhythms in insects. Here we show that a short-period mutant brain can produce a short-period activity rhythm when implanted into the abdomen of a genetically arrhythmic host. In this instance, there were no functional neural connections between the implanted brain and the locomotor system of the recipient, so the action of the brain must be mediated by humoral influences.

Adult brains from short-period mutant (*per^s*) animals which normally exhibit 18- to 20-h activity rhythms in constant environmental conditions¹³ were transplanted into the abdomens of arrhythmic mutant (*per⁰*) adults whose activity in these conditions is aperiodic. Of 55 individuals surviving the operation, 4 showed short-period activity rhythms for at least three consecutive cycles (four activity bursts) (Table 1). Figure 1a shows the activity of the A-21-24 arrhythmic host containing a

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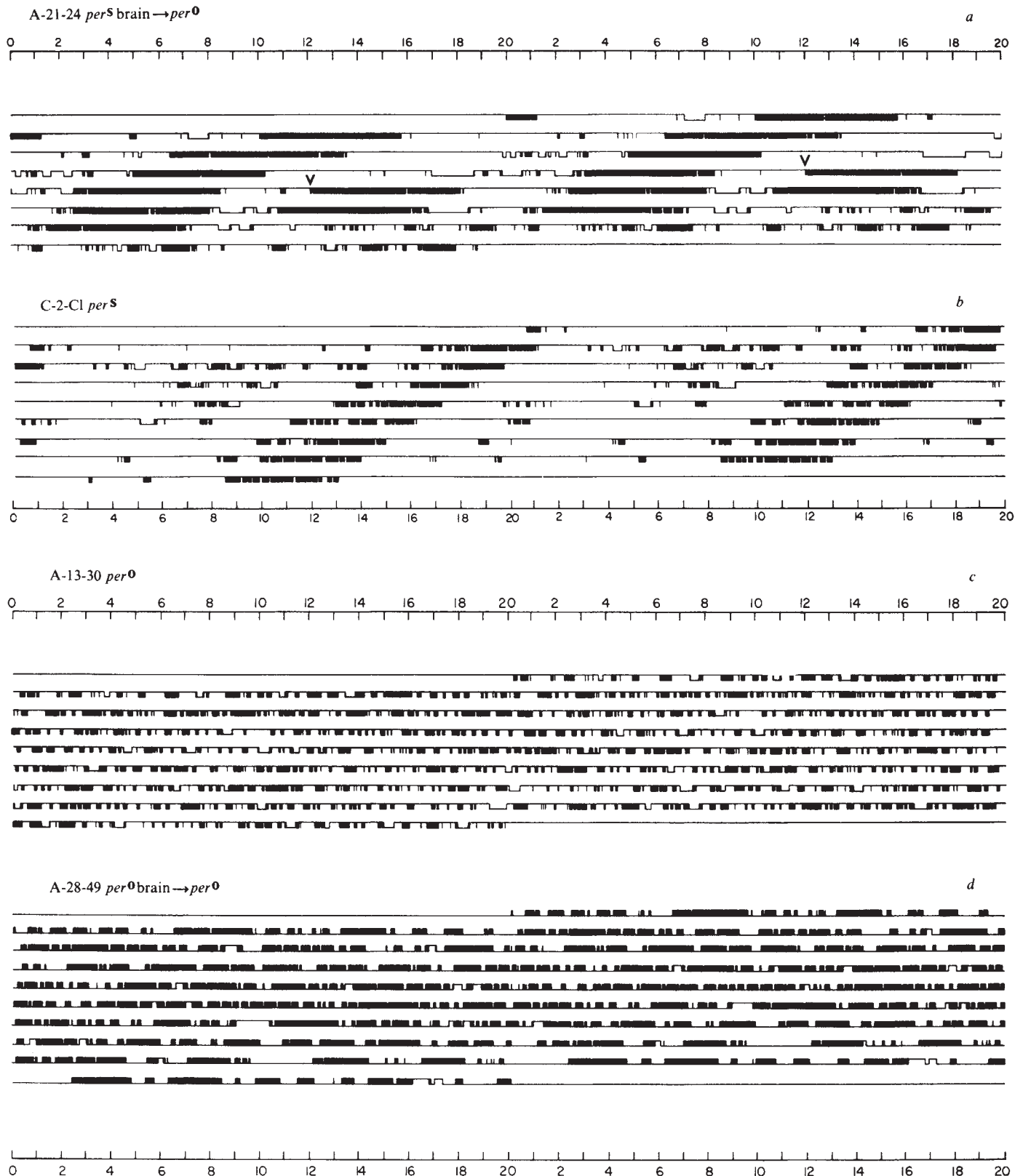


Fig. 1 Locomotor activity of individual adult *Drosophila melanogaster*. *a*, A *per⁰* fly implanted with a *per^S* brain; *b*, an unoperated *per^S* fly; *c*, an unoperated *per⁰* fly; *d*, a *per⁰* fly implanted with a *per⁰* brain. Event recorder data of activity monitored in IR light using a device similar to that described previously¹³. Data plotted modulo 20 h. Each line indicates the start of successive 20-h intervals. Successive intervals are also plotted to the right of the immediately preceding interval for visual continuity. Female flies bearing the *per⁰* or *per^S* mutation were raised in identical 12 h white light–12 h dark cycles. One to two days after eclosion, brains from *per⁰* and *per^S* donors were dissected in *Drosophila* Ringer's solution¹⁵ and injected with Ringer's into the abdomens of *per⁰* hosts, using a glass micropipette with a small constriction. Host animals were kept in a moist environment for 1 d to facilitate wound healing. Two to four days later, surviving hosts and unoperated *per⁰* and *per^S* controls which had been raised in the same light–dark cycle were inserted into the activity monitor. Locomotor activity was usually recorded for an initial 2-d entrainment period which continued the previous light–dark cycle, using long-wave UV light. Flies were then maintained in constant conditions (24 °C) for an additional 7 to 10 d during which locomotor activity was monitored in IR light. Events were also counted each hour and the digital data used for periodogram analysis.

Table 1 Activity rhythms in *per*^o hosts implanted with a *per*^s brain

Host	No. of cycles	Average period* (h)
A-21-24	6	17
A-23-79	5	16
A-23-76	4	10 and 20
A-23-27	3	18

* Average period obtained by periodogram analysis of digital activity data.

per^s brain in its abdomen. A clear short-period rhythm persists for five cycles, with a sixth cycle that is less clear. (An additional activity component with a similar period appears briefly in the record, shown by the arrow; the beginning of this activity burst coincided with a food change and probably resulted from the mechanical disturbance or the stimulus of the fresh food.) The period of the activity onsets and offsets increases slightly during the course of the record, possibly because of degenerative changes in the implanted brain. The end of the first clear activity burst occurs 69 h after the end of the last entraining light pulse, consistent with the proposition that the phase of the rhythm was set by the last light signal seen by the donor animal.

In addition to A-21-24, three other *per*^o hosts with implanted *per*^s brains showed short-period rhythmicity (Table 1). One of these, A-23-76, showed evidence of a bimodal rhythm. Activity rhythms of wild-type and *per*^s flies are typically bimodal in a light-dark cycle¹⁴ and occasionally bimodal in constant environmental conditions (R. K., unpublished results).

Figure 1b shows the activity rhythm of an unoperated *per*^s fly for comparison. In addition to the similarity in periodicity of the rhythms in Fig. 1a and b, the duration of the activity bursts (5–7 h) in both records is typical of the reduced activity span in *per*^s mutants compared with wild-type (10–13 h). Unoperated *per*^o flies were always aperiodic (Fig. 1c)^{13,14}. Furthermore, none of the *per*^o hosts containing implanted *per*^o brains showed any evidence of persistent rhythmicity ($n = 48$, Fig. 1d). Therefore implantation of extra brain tissue is not in itself sufficient for rhythmic activity.

After the activity of the *per*^o hosts implanted with *per*^s brains had been measured, surviving animals were dissected and the transplanted brains examined. Transplanted brains were morphologically recognisable in ~70% of surviving hosts 2 weeks after the operation. The remaining donor brains probably degenerated soon after the operation. Eleven donor and host brains from animals whose activity was not monitored were stained using a silver impregnation technique 2 to 4 weeks after the operation and examined under the light microscope for degeneration. All donor brains and some host brains showed some degree of cell death and neuropil degeneration, resulting in vacuolation of the tissue. The low percentage of transplants showing short-period activity rhythms may be due in large part to degeneration which ensues after dissection and implantation.

We conclude that a brain isolated from a short-period mutant fly can produce its characteristic activity rhythm when implanted into a genetically arrhythmic host. It is highly unlikely that the donor brain in the abdomen could establish functional neural connections with the host nervous system in the thorax during these brief experiments, so the conferred rhythmicity must be due to a diffusible humoral factor secreted from the donor brain. The factor then acts on the nervous system of the host, either directly on the motor centres or indirectly via the host brain, to elicit the locomotor activity rhythm. The induced activity rhythms in successful transplants were of short-period and not wild-type, so it is unlikely that the donor brain somehow complemented the defect in the host's arrhythmic pacemaker system, allowing it to function normally. The fact that activity records of successful transplants do not show aperiodic background activity at all times indicates that the factor from the implanted *per*^s brain is inhibitory, limiting the activity of the *per*^o host to discrete periodic intervals, with an absence of activity during intervening periods.

The demonstration of a humoral component in the control of *Drosophila* activity rhythms does not, of course, rule out the possibility of an additional neuronal pathway in intact flies.

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ALFRED M. HANDLER

RONALD J. KONOPKA

Division of Biology 216-76,
California Institute of Technology,
Pasadena, California 91125

Received 29 January; accepted 26 March 1979.

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Androgen-insensitive rats are defeminised by their testes

SOME chromosomal XY male humans¹, mice^{2,3} and rats⁴ are born with a vagina, labia and clitoris instead of scrotum and penis. The expression of the female phenotype in these individuals is thought to be due to an insensitivity to androgenic hormones which are secreted by the testes. This phenomenon has been termed the androgen-insensitivity syndrome or testicular feminisation. Typically, androgen-insensitive humans are identified and adopt a female gender identity⁵. Androgen-insensitive rats, on the other hand, seem to be incapable of showing a high degree of feminine behaviour in adulthood even when given oestrogen and progesterone^{6,7}. It is shown here that androgen-insensitive male rats are capable of responding to ovarian hormones with the display of feminine sexual behaviour if they are castrated at birth but not when castrated 10 d after birth. Although these rats are insensitive to their testicular secretions in terms of genital differentiation, the neural substrate of sexual behaviour is differentiated by the presence of testes during the first few days after birth.

Androgen-insensitive male rats of the Stanley-Gumbreck strain ($n = 16$) and their male littermates ($n = 19$) were castrated within 24 h of birth or 10 d after birth at the International Foundation for the Study of Rat Genetics and Rodent Control (Introgen), Oklahoma City. At weaning, the rats were sent to our animal facilities and were maintained in pairs on a reversed light-dark cycle with *ad libitum* food and water.

At 75–98 d of age, the subjects were administered 2 µg of oestradiol benzoate 48 and 24 h before injection of 500 µg of progesterone; testing began 3–4 h after the progesterone injection. Each subject was placed in a glass observation cage with a sexually vigorous male rat. The male was allowed to mount the subject 10 times. The occurrence of each lordotic response was recorded and a lordosis quotient (LQ) was determined ((number of lordotic responses per mount with thrusts) × 100). Each subject was tested twice during a 2-week period.

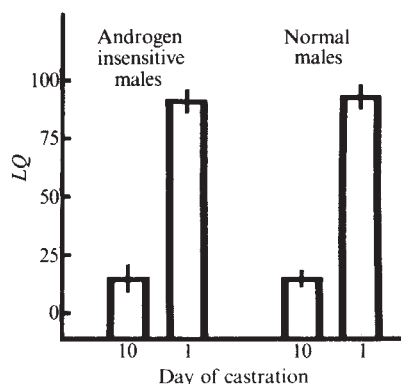


Fig. 1 Female sexual behaviour in androgen-insensitive (Tfm) and normal male rats castrated on the day of birth (day 1) or 10 d after birth (day 10) and treated with oestrogen and progesterone as adults. Receptivity was assessed by the lordosis quotient (LQ), the ratio of lordotic responses to mounts by the male.

Both androgen-insensitive and normal males castrated on the day of birth showed high levels of female sexual behaviour following oestrogen and progesterone treatment in adulthood. The mean LQ scores $\pm$ s.e.m. (and the individual range of responses) were, respectively, 90.8 ± 4.5 (75–100) and 93.6 ± 3.4 (75–100) for androgen-insensitive and normal rats. Castration on day 10 resulted in reduced mating for both groups of male rats. The mean LQ scores $\pm$ s.e.m. (and the individual range of responses) for the rats castrated after the sensitive period were, respectively, 15.0 ± 5.5 (0–60) and 14.6 ± 3.1 (0–35) for androgen-insensitive and normal rats. An analysis of variance indicated that 'day of castration' was statistically significant for both androgen-insensitive ($F = 90.9$, $df = 1, 14$, $P < 0.001$) and normal male rats ($F = 267.3$, $df = 1, 17$, $P < 0.001$). It is apparent that the behavioural response of androgen-insensitive males is like that of normal male rats.

These results clearly show that the presence of testes during the first 10 d after birth alters the later responsiveness of both normal and androgen-insensitive male rats to oestrogen and progesterone. As these animals seem to be insensitive to androgen, it is possible that this change is brought about by oestrogen produced by the aromatisation of androgen. Indeed, it has been suggested that sexual differentiation of the brain is controlled by oestrogen rather than androgen⁸ and there are several lines of evidence consistent with this hypothesis. Thus, androgen is capable of being aromatised to oestrogen in neural tissues of neonatal rats in both *in vitro* and *in vivo* conditions^{9–11}. Oestrogen binds to both cytosol and nuclear fractions of neonatal rat brains¹². The administration of oestrogen and aromatisable androgens within the first few days after birth defeminises female and castrated male rats^{13,14}. Dihydrotestosterone, an A-ring reduced androgen which is not readily aromatised, is ineffective in suppressing lordosis behaviour in rats¹⁵. Drugs that delay or prevent aromatisation attenuate the inhibition of female mating behaviour that would normally be brought about by the testes or injection of testosterone propionate^{16–18}. Oestrogen antagonists, which disrupt the action of oestrogen in neural tissue, will reduce the defeminising effect of androgen¹⁸.

The data in Fig. 1 are consistent with the 'aromatisation hypothesis', as androgen-insensitive rodents can aromatise androgens¹⁹ and do possess normal levels of neural oestrogen receptors²⁰.

Another aspect of male–female differences in neuroendocrine function is the ability of oestrogen to induce the release of luteinising hormones (LH). The administration of oestrogen to normal females causes a surge of LH whereas this positive feedback effect is absent in males. Androgen-insensitive humans are like normal males, rather than females, as they do not release LH following oestrogen injection^{21,22}.

The present results and the recent data on humans^{21,22} indicate that both androgen-insensitive humans and rats are clearly distinguishable from normal females in neuroendocrine function. Note, however, that feminine characteristics do exist, as some androgen-insensitive humans, when raised as girls, develop feminine gender role behaviour and some androgen-insensitive rats show the typical female preference for saccharine²³. These observations may still have important clinical implications for the treatment of genitally anomalous children, particularly because some males with incomplete androgen insensitivity seem to be capable of showing masculine development at puberty^{24,25}.

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K. L. OLSEN*

Department of Psychobiology,
University of California,
Irvine, California 92717

Received 11 December 1978; accepted 15 March 1979.

* Present address: Department of Neuroscience, Children's Hospital Medical Center, 300 Longwood Avenue, Boston, Massachusetts 02115

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Cytoplasmic microtubules of higher plant cells visualised with anti-tubulin antibodies

It has been known for some time from electron microscopy that eukaryotic cells contain a fibrillar cytoskeleton. Recently, immunofluorescence has greatly advanced our understanding of how the cytoskeleton functions in controlling the shape and behaviour of cells¹ by allowing immunological identification and by displaying the three-dimensional distribution of the constituent proteins. As plant cell walls exclude antibodies, such work has been carried out almost entirely on animal cells. It is not possible to extrapolate from animals to plants because the problems are not the same: plant microtubules occur in cortical hoops at right angles to the axis of cell elongation unlike, say, neuronal outgrowth; they overlap to form bundles; they do not appear to grow from focal points around the nucleus and they are conspicuously cross-bridged to each other and to the plasma membrane². In a rare study on higher plant cells, Franke *et al.*³

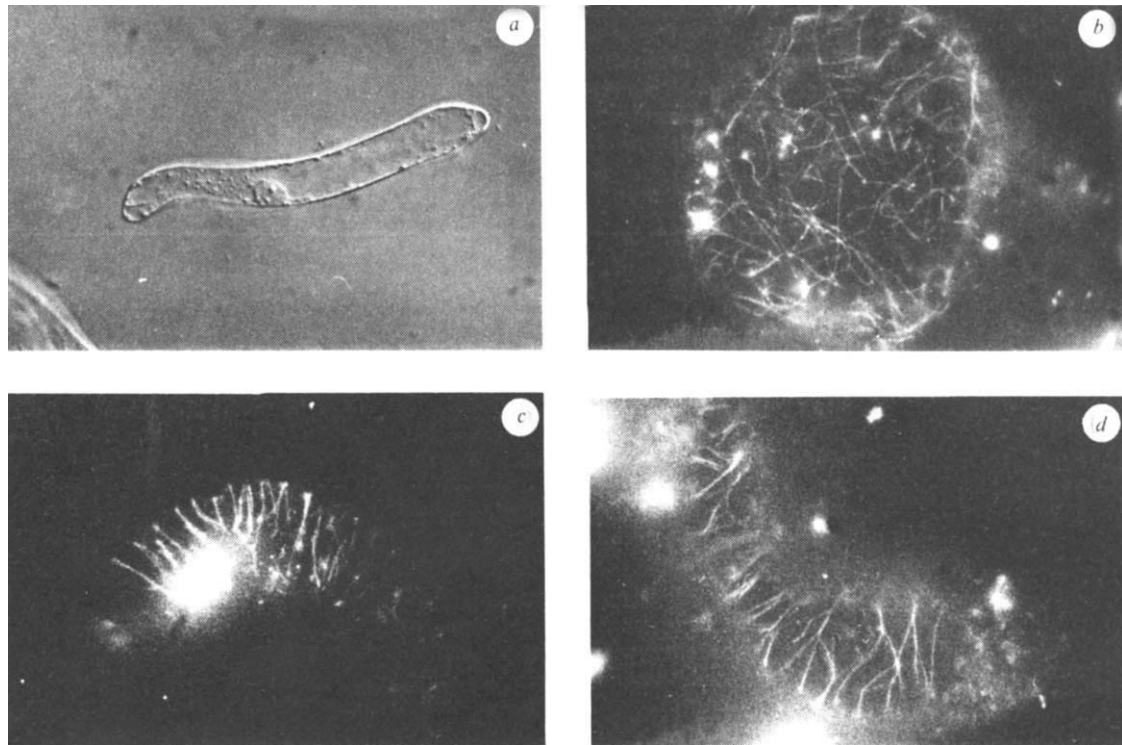


Fig. 1 Cells were grown in Murashige & Skoog's medium (GIBCO) containing 1 p.p.m. 2,4-D, 3% sucrose, 5% coconut milk and 0.5 p.p.m. kinetin. To produce elongated cells, suspensions were cultured for 6 d in medium lacking 2,4-D. Protoplasts were prepared using Onozuka R-10 cellulase (Kinki Yakult Mnfng Co.) purified on a Sephadex G-75 column and dissolved in growth medium plus 0.25 M sorbitol. Cells were treated for up to an hour to produce rounded protoplasts or where elongated cells were taken for staining they were treated for about 30 min or before significant numbers of protoplasts formed. These were collected by centrifugation at 100g, attached to coverslips pre-treated with polylysine at 1 mg ml⁻¹ and extracted in isotonic microtubule-stabilising buffer (MSB) [100 mM PIPES pH 6.9; 1 mM MgSO₄; 2 mM EGTA; 0.25 M sorbitol containing 1% Triton X-100]. After 20 min they were washed three times, fixed in 3.5% formaldehyde (10 min) and excess aldehyde neutralised with 0.1 M NH₄Cl. The preparation and specificity of the anti-tubulin antiserum have been described previously⁵. It produced a single diffusion line against the tubulin used as antigen and yielded the characteristic lace-like pattern of microtubules when used to stain rat embryo fibroblasts. Carrot cells and protoplasts were incubated for 45 min at 22 °C with anti-tubulin diluted 20 times in MSB, washed three times with MSB, incubated for 45 min at 22 °C with fluorescein-labelled goat anti-rabbit IgG antibodies (Miles) diluted 30 times in MSB and finally washed in this same buffer. Immune serum was absorbed against tubulin prepared from rabbit brains⁶. Affinity purified anti-tubulin was prepared on a column of bovine brain tubulin⁵ bound to Sepharose 4B as described previously⁷ and used at 100 µg ml⁻¹. Cells were mounted and viewed, as previously outlined⁵. *a*, Elongated carrot cell from stationary phase culture. Interference contrast, $\times 300$. *b*, Unravelled 'fibrils' in a cell converted to a protoplast by 60-min treatment with cellulase and then stained with anti-tubulin by indirect immunofluorescence, $\times 740$. *c*, *d*, Elongated cells treated for 30 min with cellulase, then extracted and stained with anti-tubulin. The 'fibrils' run around the cell in transverse, interconnected hoops, $\times 740$.

made use of the immunological cross-reactivity between mammalian anti-tubulin and plant microtubules to study the giant mitotic apparatus in the endosperm of the March cup flower. However, such temporary nutritive tissue may have no significant wall deposition and although ideal for studying the nucleus is unsuitable for studying the cytoplasmic microtubules involved with cellulose in controlling plant cell shape. Franke *et al.*³ saw no cytoplasmic microtubules and for the study reported here we therefore used single, elongated cells from carrot suspension cultures which we know from electron microscopy (EM) studies (unpublished) to contain cytoplasmic microtubules. A controlled cellulase treatment and detergent extraction to allow the entry of antibodies to these cells was used, and we believe this to be the first report of the immunofluorescent staining of cytoplasmic microtubules in higher plant cells.

Rapidly-dividing suspension cultures of carrot cells (*Daucus carota* L.) contain small clusters of roughly isodiametric cells but when allowed to grow into stationary phase or when subcultured into medium lacking the auxin (2,4-dichlorophenoxy)acetic acid (2,4-D), the cells stop dividing and begin to elongate (Fig. 1*a*). This is the simple cell model with which we intend to study the role of microtubules in shape control and the development of axial polarity. Two sorts of enzyme treatment were used to degrade cell walls: (1) an extended incubation in cellulase which converts most cells to spherical protoplasts (which allows more reproducible antibody staining and is used for controls); and (2) a brief enzyme treatment which does not affect the

elongated cell shape. Treated cells were attached to polylysine-treated coverslips and then extracted in isotonic microtubule-stabilising buffer containing Triton X-100 which reduced background fluorescence and allowed penetration by antibodies. Fixing cells with formaldehyde before acetone or detergent extraction was not successful. Detergent-resistant material was then stained with rabbit antibodies raised against bovine brain tubulin and visualised by adding fluorescent goat anti-rabbit antibodies. Using protoplasts, two patterns of staining predominate: a circular, compressed 'footprint' composed of fibrils (Fig. 1*b*) and a more substantial structure of fibrils often containing the nucleus. However, if immune serum is substituted by pre-immune serum, such fibres are only rarely stained, and then only weakly. Absorbing the immune serum with rabbit brain tubulin abolishes the typically intense staining of fibrils; the FITC-conjugated goat anti-rabbit antibodies when used alone do not stain any fibrillar material. As a further check, anti-tubulin purified on a tubulin affinity column has been used and this also stains fibrils intensely.

These results are therefore consistent with the presence of a tubulin-containing network in carrot protoplasts. An obvious candidate for such a network would be the microtubules we have seen by EM (not shown) in cells and protoplasts, except that patterns of microtubules which are characteristic of elongated cells are undoubtedly modified as the cells form protoplasts. Our unpublished electron micrographs confirm, as others have shown², that elongated plant cells possess girdles of micro-

tubules, and that to examine this better-defined arrangement it is essential to treat cells with cellulase for a long enough period to allow penetration of antibodies but not so long as to degrade shape and alter the pattern of microtubules. Careful monitoring of the progress of cellulase treatment is therefore required. When treated as described with anti-tubulin, elongated cells show fibrils at right angles to the cell's long axis (Fig. 1c, d) and differential focusing indicates that these encircle the cell and branch. Plant microtubules are cross-bridged and so these fluorescent images are consistent with the presence of branched bundles of microtubules which form interconnected, transverse hoops along the entire cell length. When the walls are removed by cellulases, elongated cells become spherical even though they still contain microtubules. Conversely, colchicine-treated cells become spherical even though they possess a cell wall (not shown). This suggests that external cellulose and internal microtubules somehow combine to control plant cell shape, and a transmembrane model connecting the two systems has been proposed⁴. By showing that fluorescent patches of lectin on the surfaces of animal cells run parallel to rhodamine anti-myosin-stained intracellular filaments, Ash and Singer¹ have pointed towards a transmembrane control in animal cells. The demonstration here of cortical microtubules by immunofluorescence displays the special distribution of these elements in higher plant cells and suggests new ways in which the presumed role of microtubules in influencing plant cell shape can be tested.

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CLIVE W. LLOYD*
ANTONI R. SLABAS
A. J. POWELL
G. MACDONALD
R. A. BADLEY

Biosciences Division,
Unilever Research,
Sharnbrook, Bedford, UK

Received 9 March; accepted 12 April 1979.

* To whom correspondence should be addressed.

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Cigarette smoke condensates damage DNA in human lymphocytes

THE carcinogenicity of tobacco tars and smoke in laboratory animals^{1,2} together with epidemiological evidence from man have clearly suggested that smoking causes most lung cancer³⁻⁵. However, other interpretations of the epidemiological evidence have been proposed⁶ and the carcinogens known to occur in cigarette smoke are present in only minute quantities⁷. There is increasing evidence that one prerequisite in the initiation of malignant transformation is an alteration in cellular DNA, and the findings that most carcinogens react with DNA⁸, that this reaction necessarily precedes transformation⁹ and that most carcinogens are mutagens¹⁰, support the importance of somatic mutation. Two types of visible chromosome changes that result from DNA damage are gross aberrations and symmetrical sister chromatid exchanges (SCE)^{11,12}, and the induction of SCEs provides a very sensitive indicator of mutagen/carcinogen exposure at concentrations below those which normally result in the production of chromosomal aberrations^{13,14}. Cytogenetic

Table 1 Cigarette condensate yields

Tar category of cigarette	Average dimensions of cigarette		Butt length (mm)	Weight of condensate (wet in mg) collected from 24 cigarettes smoked together*
	Length (mm)	Weight (mg)		
High	70	1,008	20	1,020
Middle	70	988	20	530
Low	83	1,012	25	270
	(including 22-mm filter)		(including filter)	

* Each cigarette received, in turn, puffs of 35 ml lasting 2 s at a frequency of once per minute until the desired butt length was reached.

studies^{15,16} have shown an increase in chromosomal aberrations in blood lymphocytes of heavy smokers relative to non-smokers, but little¹⁷ or no^{16,18} increase in SCEs. Furthermore, products mutagenic to bacteria have been isolated from the urine of inhaling smokers¹⁹, and also smokers have a higher incidence of sperm abnormalities than non-smokers²⁰. These studies imply that there may be higher levels of mutagens in body cells and fluids of smokers than in those of non-smokers, but little is known about the reaction of cigarette smoke with the DNA of exposed human cells, and even less about the possible mutagenic effects. Here we report that exposure of human lymphocytes to small quantities of tobacco smoke condensate leads to the formation of many DNA lesions that result in sister chromatid exchanges.

Smoke condensates were prepared, by standard methods²¹ using an automatic smoking machine and impaction trap, from three popular brands of British cigarettes of low, middle and high tar content (Table 1). The condensates from each brand were wet weighed and, having similar solubilities, were dissolved in dimethyl sulphoxide (DMSO, 50 mg ml⁻¹) and further diluted with sterile distilled water to give final condensate solutions (5 mg ml⁻¹).

Cultures were set up with lymphocytes from heparinised venous blood of two healthy non-smoking males, using a whole blood microculture technique²². Eleven cultures (final volume 10 ml) were set up from each blood sample and all contained 0.8 ml blood in RPMI 1640 medium (GIBCO) with fetal calf serum (16.6%), glutamine (0.8%), phytohaemagglutinin (1%), penicillin (100 U), streptomycin (100 µg) and 5-bromodeoxyuridine (BUdR, 25 µM). One culture from each subject was untreated and a second received 0.5 ml of the diluent (DMSO and water 1:9). The remaining nine cultures from each donor

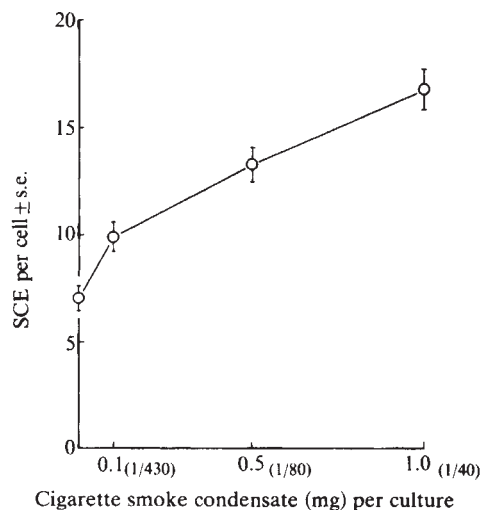


Fig. 1 SCE frequencies (means $\pm$ s.e.m. from 20 metaphases per culture) in lymphocytes exposed to smoke condensate from high tar cigarettes. The abscissa shows mg of condensate added to each 10-ml culture containing 10^6 lymphocytes and the amount of smoke from one high tar cigarette required to produce that amount of condensate.

Table 2 Frequency of sister chromatid exchanges in blood lymphocytes exposed to smoke condensates from low, middle and high tar cigarettes

Dose of smoke condensate per 10-ml culture	Donor	Control	Control with DMSO	Cigarette brand		
				Low tar	Middle tar	High tar
0	A	5.35 ± 0.57	6.05 ± 0.64			
	B	5.15 ± 0.51	5.00 ± 0.44			
0.1 mg	A			8.50 ± 0.65	8.55 ± 0.52	8.25 ± 0.50
	B			8.65 ± 0.66	7.95 ± 0.65	9.30 ± 0.68
0.5 mg	A			13.25 ± 0.69	14.30 ± 0.78	13.50 ± 0.78
	B			15.00 ± 0.71	14.15 ± 0.79	13.65 ± 0.76
2.5 mg*	A			—	—	—
	B			—	—	—

Frequency is the mean ± s.e. from 20 metaphases per sample.

* Cytotoxic dose level, no metaphases in culture.

were divided into three groups for treatment with condensate from low, middle and high tar cigarettes at concentrations of 0.1, 0.5 and 2.5 mg of condensate per culture. All cultures were grown for 72 h in the dark (each receiving colcemid solution in the final 3 h), collected, fixed, and air-dried preparations made. The preparations were stained by a slightly modified version of the BUdR-Hoechst 33258-Giemsa method²³ and all slides were coded before scoring.

No cytotoxicity or difference in SCE frequency were noted between donors or control cultures exposed or not exposed to 0.5 ml DMSO/water solvent. In cultures from both subjects 2.5 mg of condensate from each of the three brands of cigarettes was cytotoxic, whereas smaller amounts gave dose-dependently increased SCE frequencies significantly above control values (Table 2). Of course, the amounts of condensate produced by the low, middle and high tar cigarettes differed (Table 1), but equivalent amounts of tar from the three brands induced equal numbers of SCEs. The pooled data show that exposure of a 10 ml-culture containing 1×10^6 lymphocytes to 0.5 mg of condensate results in an SCE frequency two- to threefold that in controls. We have obtained similar results in several repeat experiments on blood cells from a series of individuals and using tar preparations stored for up to 1 month at -20°C . Figure 1 shows a typical response.

Our findings show that cigarette smoke condensates, produced by a cigarette-smoking machine, interact with the DNA in human cells *in vitro* and that this interaction can be detected at exposure levels that seem surprisingly small: the SCE frequency was detectably increased by 0.1 mg of condensate and more than doubled by 0.5 mg, these doses representing 1/400th and 1/80th, respectively, of a single high tar cigarette. In terms of equivalence of effect, the yield of SCE following exposure to 0.5 mg of condensate is equal to that obtained after exposure of human lymphocytes to the powerful mutagens aflatoxin B₁ and mitomycin C at concentrations of 10^{-4} M and 5×10^{-8} M, respectively (unpublished data).

A range of precarcinogens, carcinogens, cocarcinogens and promoters has been identified in cigarette smoke^{7,24} and the polynuclear aromatic hydrocarbons, particularly the eventual metabolites of benzo(a)pyrene (BP), are regarded as the most potent carcinogens⁷. We, however, find that 2.5 µg of BP (an amount equivalent to that in the smoke of 50–250 cigarettes²⁴) is required to produce an increase in SCE similar to that due to 0.5 mg of smoke condensate (1/80th of a cigarette). This demonstrates that the activity of tobacco smoke condensate in producing the DNA lesions that result in SCE cannot be ascribed to its BP content alone and this parallels the findings that the total carcinogenic activity of tobacco smoke tars in animals cannot be explained by a single class of compounds, or by the simple additive effects of the various known constituents⁷. Similarly, recent studies²⁵ in bacteria on the mutagenicity of pyrolysed food products have also demonstrated that the BP content is insufficient to account for even a small fraction of the mutagenicity, but that pyrolysed amino acids, and in particular tryptophan, are extremely potent bacterial mutagens.

Finally, we emphasise that although there may be qualitative similarities between the response to tobacco tar of lymphocytes *in vitro* and bronchial epithelium *in vivo*, we cannot know whether the cells respond in the same way or to the same degree. Furthermore, although the SCEs we observe in the chromosomes of exposed cells reflect induced DNA damage, these may not in themselves be mutational events, although their incidence in mammalian cells in culture is certainly correlated with the incidence of mutations²⁶. Nevertheless, our results show that the levels of agents that can react with cellular DNA may be particularly high in the respiratory airways of inhaling cigarette smokers, in keeping with the somatic mutational theory of malignant transformation and the epidemiological evidence that many cigarette smokers ultimately die of lung cancer. In this latter context our system may be useful for examining variations in responsiveness to smoke amongst humans²⁷ and we are exploring this possibility.

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J. M. HOPKIN
H. J. EVANS

Medical Research Council,
Clinical and Population Cytogenetics Unit,
Western General Hospital,
Edinburgh, UK

Received 1 February; accepted 6 April 1979.

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A small polypeptide different from β_2 -microglobulin associated with a human cell surface antigen

A STRIKING structural feature of several cell surface molecules in warm-blooded vertebrates is the non-covalent association of polypeptide chains (molecular weight (MW) 40–45,000) bearing alloantigenic determinants, with another molecule, β_2 -microglobulin (β_2 m) (MW 11–12,000)^{1–4}. A role for β_2 m has not been established, but a whole family of cell surface proteins with overall structural similarity to major transplantation antigens have β_2 m as a common invariant subunit. The identification of β_2 m in these proteins has relied mainly on its characteristically low MW and on the use of heterologous antisera. Human thymus antigen (HTA 1) is a surface antigen found on human thymocytes and certain thymomas like MOLT-4. It has been identified by the monoclonal antibody from hybrid myeloma NA1/34 and has been suggested to represent the human homologue of the murine TL antigen⁵. We describe here experiments showing that the heavy chain of HTA1 is associated with a ' β_2 m-like' subunit which is different from β_2 m both in its mobility in SDS polyacrylamide gel electrophoresis (PAGE) and in its immunological properties.

MOLT-4 cells⁶ were radioactively labelled using lactoperoxidase-catalysed iodination, lysed in Nonidet P-40, and the dialysed lysate used for immunoprecipitations⁷. In some cases aliquots of the lysate were also passed over immunoabsorbents.

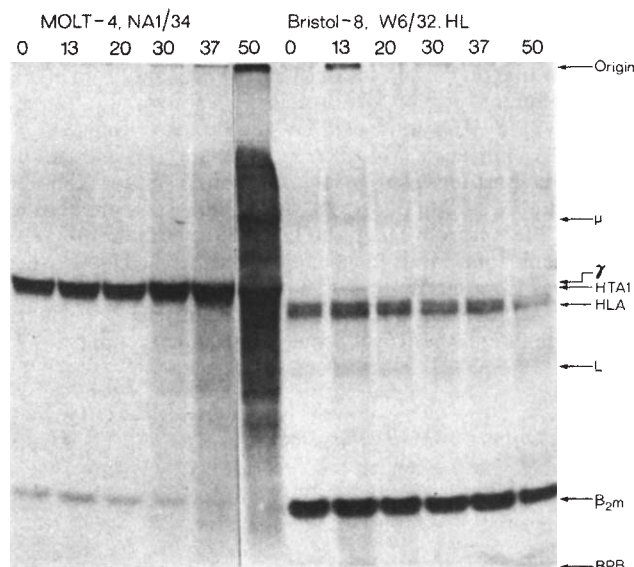


Fig. 1 Analysis by SDS-PAGE in reducing conditions of immunoprecipitates made with NA1/34 from ¹²⁵I-MOLT-4 lysates or W6/32.HL from ¹²⁵I-Bristol-8 lysates. [W6/32.HL and W6/32.HK are newly derived clones from the hybrid myeloma line W6/32.HLK described by Barnstable *et al.*²⁰. The active variant W6/32.HL retains the specific heavy and light chains of the original hybrid, but W6/32.HK is an inactive variant containing the specific heavy chain in association with the myeloma light chain (unpublished results).] The 7% acrylamide gel was as described previously¹⁹ but also contained 3 M urea. Aliquots (100 μl) of the lysates were incubated at 0, 13, 20, 30, 37 and 50 °C with equal amounts of either NA1/34 or W6/32.HL culture supernatant for 1 h, then an optimal amount of sheep anti-P3 serum was added followed, after 10 min further incubation at the respective temperatures, by 10 μl normal mouse serum. Mixtures were kept for 18 h at 4 °C and then processed as described before⁷. The autoradiograph was exposed for 14 days (first 5 slots from the left) or 5 days with an intensifying screen²¹. Internal MW markers were μ -chains (MW 75,000), γ -chains (MW 50,000) and L-chains (MW ~23,000). Bromophenol blue (BPB) served as front marker. The positions of HTA1 heavy chain, HLA-heavy chain and β_2 m are also indicated.

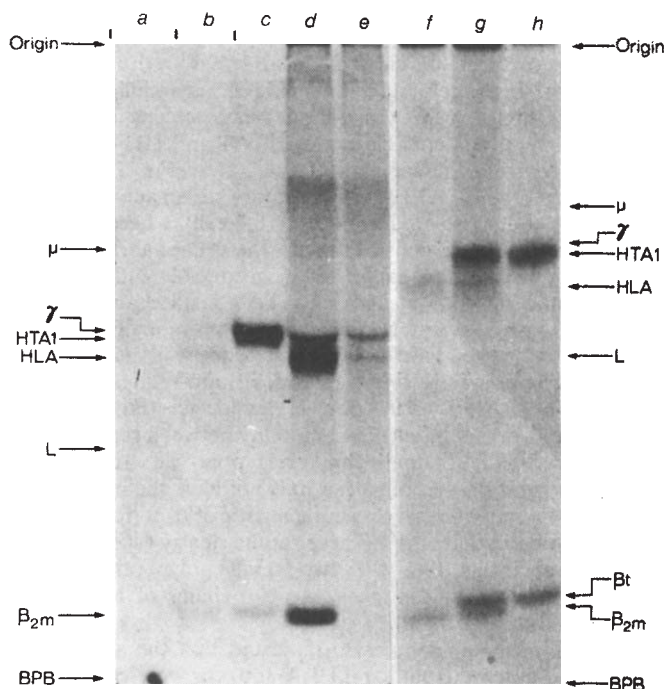


Fig. 2 Analysis by SDS-PAGE of immunoprecipitates and immunoabsorbent eluates from a ¹²⁵I-MOLT-4 lysate on 7% (slots a-e) or 10% (slots f-h) acrylamide gels as in Fig. 1. Aliquots (100 μl) of lysate were incubated for 18 h at 4 °C with 100 μl of W6/32.HK (control) (a), W6/32.HL (b), NA1/34 (c), 5 μl goat anti-human β_2 m (d) or 5 μl normal goat serum (e). Immunoprecipitates were generated by addition of anti-P3 and normal mouse sera (a-c; see Fig. 1) or pig anti-'ruminant' Ig serum (d, e; see ref. 20 for details). Aliquots of washed immunoprecipitates were subjected to SDS-PAGE in reducing conditions (slots a-e). For immunoabsorption, aliquots of the same ¹²⁵I-MOLT-4 lysate were passed five times at 4 °C over either a W6/32 or a NA1/34 immunoabsorbent. After extensive washing with phosphate-buffered saline (PBS) containing 0.5% NP40, elution was carried out with 2% SDS in PBS. Aliquots of the eluates were subjected to SDS-PAGE in non-reducing conditions: W6/32 eluate (HLA antigens) (f), mixture of W6/32 and NA1/34 eluates (g), NA1/34 eluate (HTA1 antigen) (h). The autoradiographs were exposed using intensifying screens for 14 days (slots a-e) or 10 d (slots f-h). Internal MW markers and designations of bands are as in Fig. 1. β t Shows the position of the small chain associated with HTA1 heavy chain.

Washed immunoprecipitates or column eluates were subjected to SDS-PAGE for final analysis. Earlier experiments had shown that NA1/34 antibody reacted specifically with a molecule which could be iodinated and which, after analysis by SDS-PAGE, disclosed a component of MW comparable to that of HLA heavy chains⁵. However, the presence of a small MW component was also suspected (see Discussion of ref. 8). This was because in some NA1/34 immunoprecipitates a molecule with MW comparable to that of β_2 m had been found, but only with poor reproducibility. We tested whether the temperature of the first step of the immunoprecipitations had any effect on the results (Fig. 1). For comparison, similar immunoprecipitation experiments on HLA antigens from Bristol-8 cells (a human B-cell line⁹) were performed in parallel. Higher temperatures (≥ 20 °C) led to a dissociation of the heavy and light chains precipitated by NA1/34, and only at 50 °C was there a slight reduction in the amount of both HLA heavy chains and β_2 m precipitated by W6/32.HL. We concluded that HTA1 contained two components, giving it a basic structure very similar to that of HLA antigens. Previous immunoprecipitations with NA1/34 had either been carried out at 37 °C (ref. 5) or 20 °C (A.Z., unpublished results), and in particular, the first procedure seems to be unfavourable for the detection of the small polypeptide associated with HTA1 heavy chain. All

subsequent immunoprecipitation and immunoadsorption procedures were therefore carried out at 4 °C so as to minimise dissociation. In the gel system used, HTA1 heavy chains seemed to have a higher MW than HLA heavy chains. This was confirmed by direct comparison of HTA1 and HLA antigens precipitated from the same MOLT-4 lysate (Fig. 2a-c). We estimate that the HTA1 large polypeptide has an apparent MW of about 49,000 if isolated from MOLT-4 cells, whereas that of HLA heavy chains is consistent with the usual value of about 43,000. A hardly noticeable, but reproducible difference in mobility of reduced β_2m and the HTA1 small chain was observed in these gels: β_2m seemed to migrate slightly faster than the other molecule. This mobility difference was increased by subjecting immunoadsorbent eluates, in non-reducing conditions, to SDS-PAGE in 10% polyacrylamide gels (Fig. 2f-h). In contrast to β_2m , no difference in mobility between reduced and non-reduced HTA1 small chain was observed (results not shown), suggesting that the latter may not have the intra-chain S-S loop of about 60 residues characteristic of β_2m (ref. 10) and immunoglobulin domains¹¹. These results clearly established in biochemical terms that the two small polypeptides were different. As in HLA antigens, the two chains of HTA1 are non-covalently associated.

Immunological evidence also indicated that the small MW component differed from β_2m . HLA antigens were completely removed from a lysate of MOLT-4 cells by precipitation with anti- β_2m . The immunoprecipitate contained only two bands not present in controls that were in the positions of β_2m and HLA

heavy chains (Fig. 2d, e); no radioactivity above control was precipitated in the region of the HTA1 heavy chain. When the supernatant of such anti- β_2m precipitation (from which no further β_2m could be precipitated) was reprecipitated with NA1/34, the two bands characteristic of HTA1 were easily obtained (Fig. 3). Controls in which normal goat serum (NGS) was used instead of anti- β_2m for the immunoprecipitations were also carried out (Fig. 3).

These experiments indicate that β_2m and the small chain of HTA1, which we propose to name β_t , are immunologically and biochemically distinct, and may be completely different. It seems unlikely that post-translational modifications are responsible for the observed differences. A study of the β_t amino acid sequence would be required to establish to what degree these two small molecules are different. TL antigens, the possible mouse homologue of HTA1, consist of two non-covalently bound polypeptide chains with MWs of ~45,000 and 11–12,000 (ref. 12). Several groups have reported that the small chain associated with the TL heavy chain is mouse β_2m (refs 13–15). These data rely on the specificity of the conventional polyclonal anti-TL and anti- β_2m sera used to precipitate the labelled antigens. If the anti- β_2m sera used cross-reacted with a putative mouse β_t , misleading results would have been obtained by sequential immunoprecipitations. The anti-TL sera might also be contaminated by antibodies reacting with antigens of the Qa-region¹⁶, one of which (Qa-2) has been reported to be associated with β_2m (ref. 17). The evidence presented here means either that HTA1 and TL antigen are not homologous at least as far as the small polypeptide is concerned or that the TL antigen contains β_t and not β_2m .

Our results, together with those of Tada *et al.*¹⁸ and F. M. Brodsky, W. F. Bodmer and P. Parham (unpublished results), also show that on the surface of MOLT-4 cells there are at least three different groups of proteins with overall structural similarity: the HLA antigens (MW 43,000 heavy chain + β_2m), the HTA1 antigen (MW 49,000 heavy chain + β_t) and another antigen comprising β_2m associated with a heavy chain of apparent MW ~38,000 (ref. 18) or 43,000 (A.Z., unpublished results) different from HLA and HTA1 heavy chains.

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ANDREAS ZIEGLER*
CESAR MILSTEIN

MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge, UK

Received 30 January; accepted 4 April 1979.

* Present address: Medizinische Klinik, Abteilung Innere Medizin II, Otfried-Müller-Strasse, D-7400 Tübingen 1, FRG.

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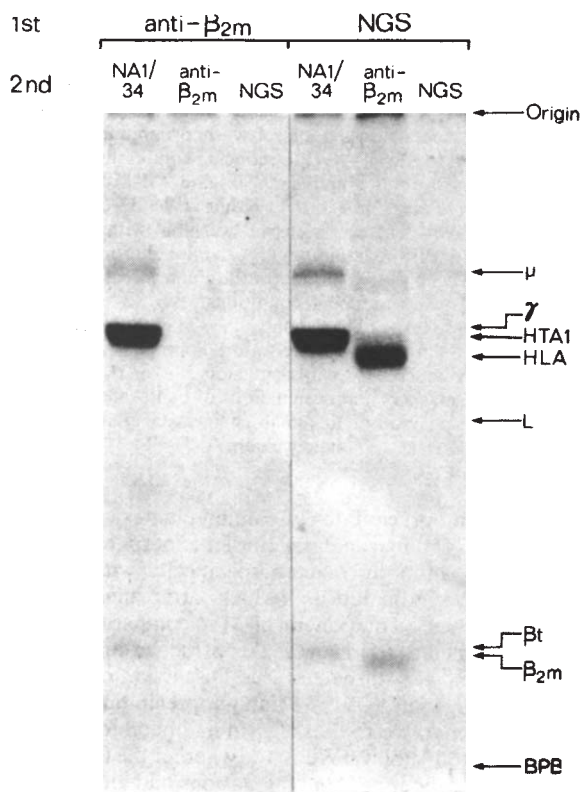


Fig. 3 Analysis by SDS-PAGE in reducing conditions of immunoprecipitates from a ¹²⁵I-MOLT-4 lysate. Lysate (100 μl) was treated at 4 °C for 18 h with 5 μl anti- β_2m serum or NGS (as control), followed by anti-‘ruminant’ Ig serum (see Fig. 2). After centrifugation, the supernatant was divided into aliquots, to which were added NA1/34 culture supernatant, anti- β_2m or normal goat serum, respectively. Samples were incubated at 4 °C for 18 h before the corresponding second antibodies (see Figs 1 and 2) were added. Aliquots of washed immunoprecipitates were subjected to SDS-PAGE on 10% gels, and the autoradiograph was exposed for 20 days with an intensifying screen. Internal MW markers and designation of bands as in Figs 1 and 2. 1st denotes sera of first precipitation, 2nd indicates supernatant and sera used for second precipitation.

Sera and cerebrospinal fluids from normal uninfected sheep contain a visna virus inhibiting factor

VISNA is a slow virus disease of sheep characterised by a long subclinical period followed by a slowly progressing clinical illness¹. The persistent visna infection is not preceded by an acute clinical phase or by rapid virus replication. This is puzzling because visna virus replicates rapidly in sheep cell cultures^{2,3}. In an attempt to resolve this paradox we inoculated visna virus into sheep cell cultures maintained in fluids with 90% sheep serum but otherwise containing the same nutrients as control cultures maintained without serum. We found that viral growth was much slower in cultures with 90% serum than in control cultures. Further experiments showed that normal sheep sera inactivate visna virus rapidly at 37 °C. We conclude that sheep sera have a visna virus inhibiting factor (VIF) and hypothesise that VIF inhibits early spread of visna virus in inoculated sheep and may be involved in initiating the persistent infection.

The sera used in experiments were drawn from the jugular vein of 6-month-old Dorset lambs. Clear cerebrospinal fluids (CSF) were obtained from the same animals by lumbar puncture. These animals were purchased from a farm in New Jersey where visna-maedi-like diseases have never been observed. Sera and spinal fluids from young Icelandic sheep were used in some experiments.

In the first experiment, sheep cell monolayers were changed to 0.5 ml of fluid containing 90% of either lamb serum or CSF in maintenance medium. Control cultures contained 0.5 ml of plain maintenance medium. Each culture was inoculated with a small amount of visna virus, thoroughly mixed with the medium. The cell layers were observed for cytopathic effect (CPE) and samples of medium were removed for titration at intervals of 1 to 2 d. The results (Fig. 1, left panel) show a total inhibition of viral growth in the presence of 90% lamb serum and an almost complete inhibition in 90% spinal fluid during a period of 7 d. No CPE was visible in either culture and the cell layers looked healthy. Larger virus inocula were less inhibited by 90% lamb serum (Fig. 1, right panel) but the inhibition was still significant. Cell layers inoculated with an input multiplicity of 2 in the presence of 90% lamb serum or CSF showed only a few scattered foci of cells with CPE on days 6 and 7, whereas cell layers maintained in 0.2% bovine serum albumin were completely disintegrated. Herpes simplex virus (HSV) type I and vaccinia virus, on the other hand, caused CPE and proliferated equally well in sheep cell cultures with or without 90% lamb serum and CSF (data not shown). Thus normal lamb serum and spinal fluid contain a visna VIF that, depending on the size of the inoculum, either prevents or greatly limits visna virus infection in cultures of sheep cells.

Preliminary experiments showed that incubation of sheep cell cultures with 90% lamb serum for 24 h before inoculation with visna virus does not inhibit the subsequent viral infection in maintenance medium. Furthermore, infected sheep cells produce virus by budding in the presence of 90% lamb serum, indicating that production of virus particles is not inhibited. We, therefore, tested the direct effect of the VIF on visna virus infectivity. Figure 2 (left panel) shows that the virus was inactivated much more rapidly in the presence of lamb serum and CSF than in maintenance medium alone. The VIF, therefore, directly inactivates the virus. The inactivation rate was particularly rapid when the virus was incubated in serum or CSF without medium added (centre panel). Although this experiment demonstrates a direct effect of VIF on the virus, an additional effect on virus-host cell interaction has not been ruled out.

We found the VIF in sera and CSF of all 10 Dorset lambs tested and also in sera and CSF from Icelandic sheep. The VIF

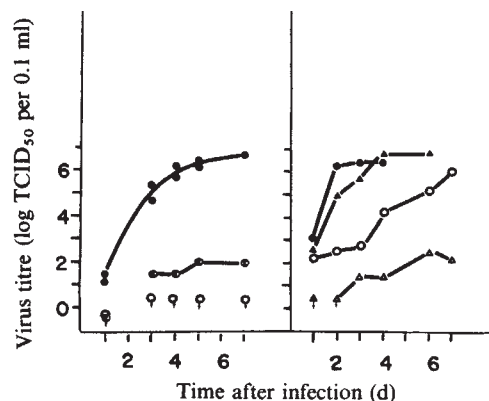


Fig. 1 Growth curves of visna virus, strain K796, in sheep choroid plexus (SCP) cells. The SCP cultures were grown in multiwell tissue culture plates in Eagle's basal medium (BME) with 15% sheep serum. Individual wells with confluent monolayers were changed to 0.5 ml of the following media: 90% lamb serum, 10% BME, 10× concentration (10×BME) (○, △); 90% lamb CSF, 10% 10×BME (●, ▲); 90% distilled water, 10% 10×BME (○, ●). All the media contained 0.2% bovine serum albumin (BSA, fraction V). Wells were inoculated with virus at an input multiplicity of 0.002 (left panel), 0.02 (right panel, △, ▲) or 2 (right panel, ○, ●) TCID₅₀ per cell. The whole fluid was collected from each well on day 1 and replaced. Thereafter, small samples of fluid were collected on the days indicated and the virus content assayed by end-point titration. The virus production was markedly reduced in cultures containing 90% lamb serum or CSF as compared with cultures in plain maintenance medium (BME with 0.2% BSA). The inhibition was particularly pronounced in cultures inoculated with small amount of virus.

did not only inactivate strain K796 but also five other strains of visna and maedi virus. All the strains were apparently inactivated at similar rates. On the other hand, measles virus, vaccinia virus, Newcastle disease virus and HSV, type I were not appreciably inactivated by undiluted lamb serum at 37 °C.

Two facts strongly indicated that the VIF is not an immunoglobulin with specific antibody against visna virus or a serologically related agent. First, the lambs had not been in contact with visna-infected sheep, and they came from areas where visna or maedi have never been found. Second, spinal fluids have a high inhibitory activity although their γ -globulin content is low. We confirmed the non-immunoglobulin nature of the VIF by precipitating the γ -globulin from lamb sera with 33% and 50% saturated ammonium sulphate, testing the activity of the three fractions against visna virus. Virus incubated with the two γ -globulin fractions in maintenance medium was inactivated at the same rate as virus incubated in maintenance medium alone. The serum supernatant that was devoid of detectable γ -globulin inactivated visna virus at a much faster rate although less rapidly than the whole serum (Fig. 2, right panel). This is in contrast to an antiserum from a visna-infected sheep, in which the antibody activity remained in the γ -globulin fraction precipitated by 33% ammonium sulphate.

The visna VIF is not dialysable or filterable through an Amicon filter (Type UM 10) and resists heating at 100 °C for at least 10 min. Preliminary tests indicate that it is resistant to chymotrypsin and α -amylase but sensitive to pronase. These results, together with a preliminary finding that an ethanol-ether extract of lamb serum had inhibitory activity, suggest that the visna VIF may be a lipoprotein. However, a biochemical identification of the VIF must await a substantial purification of the active substance.

The demonstration of a visna VIF in normal sheep sera and CSF suggests that it is of importance as a defense mechanism in the early stages of visna virus infection. The lack of an early acute phase with clinical signs, rapid virus replication, and active antibody formation becomes understandable if we assume that

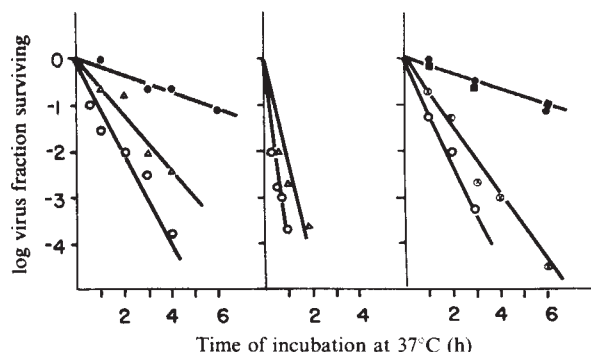


Fig. 2 Inactivation curves of visna virus diluted 10^{-1} in the following media and incubated at 37°C . Left panel: $\circ$, 90% lamb serum, 10% $10\times\text{BME}$; $\triangle$, 90% lamb CSF, 10% $10\times\text{BME}$; $\bullet$, 90% distilled water, 10% $10\times\text{BME}$ (all the media contained 0.2% BSA). Centre panel: $\circ$, 100% lamb serum; $\bullet$, 100% lamb CSF. Right panel: $\circ$, $\bullet$, same as left panel; $\otimes$, 90% lamb serum supernatant after ammonium sulphate precipitation, dialysis against distilled water and concentration to the original serum volume; $\blacksquare$ 90% lamb serum γ -globulin precipitated with 33% ammonium sulphate, partially purified by Sephadex G-200 chromatography, and dialysed against distilled water. An immunodiffusion test in agar gel, using a specific antiserum against sheep globulin, showed that the supernatant contained less than 0.25% of the serum γ -globulin, whereas the precipitate solution contained more than 50%. All the media contained 10% $10\times\text{BME}$ and 0.2% BSA. Sera and spinal fluids inactivate visna virus and the inactivation rate is very rapid if no BME is added. γ -Globulin-free serum retains most of the activity.

much of the virus inoculum is inactivated by the VIF before reaching susceptible cells and that virus produced by infected cells spreads slowly because of the presence of VIF in body fluids. The larger the visna virus inoculum the greater the likelihood that it may result in an acute infection. This agrees with an observation made by Sigurdsson in his early visna experiments in sheep⁴.

A preliminary observation supports the notion that VIF inhibits viral spread in inoculated sheep. Visna virus was inoculated into cultures of sheep fibroblasts derived from a skin biopsy from a Dorset lamb and at the time of active virus production the cells were scraped off the surface and inoculated into the eye of the same lamb. Although the eye may be considered similar to a tissue culture system in the containment of an injected antigen⁵, the virus-infected autologous cells had no detectable effect on the eye and no virus was found in a sample of vitreous fluid withdrawn a few days later.

Visna virus persists in inoculated sheep for years^{6,7} and can be recovered at intervals from buffy coat and CSF, where it is detectable in very small amounts⁸. It has been shown that visna DNA provirus is integrated into the host cell genome⁹ and that there is a block in virus replication *in vivo*, beyond the stage of provirus formation¹⁰. This provides a mechanism for persistence of visna virus in infected sheep^{10,11}. Our early work on isolation of visna virus from tissue explants of infected sheep showed that virus persisted for months in the explant cultures if 20% lamb serum was added to the medium, whereas there was a rapid virus production and cell destruction in cultures with 2% serum¹². Preliminary observations also show that 90% lamb serum inhibits viral production and CPE in sheep cell cultures that are inoculated with visna virus before addition of serum to the medium (data not shown). We, therefore, hypothesise that the VIF is of importance in restraining the persistent visna infection in sheep, at least in its early stages before the appearance of neutralising antibodies.

Although a visna VIF has not previously been demonstrated in sheep sera, the presence of visna inhibitors has been reported in human and other mammalian sera¹³⁻¹⁵. The highest neutralising activity is found in bovine sera. The non-immunoglobulin

inhibitors found in human and bovine sera are heat stable but otherwise their relationship to the sheep VIF is unknown. The same is true for various other viral inhibitors present in the sera of many animal species¹⁶⁻²². Some of them have properties similar to those of the visna VIF, although their viral spectra differ. The biological roles of the different viral inhibitors present in animal sera are unknown, but may be of great significance in determining the natural resistance to viral infection^{23,24}.

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H. THORMAR

H. M. WISNIEWSKI

F. H. LIN

*New York State Institute for Basic Research in
Mental Retardation,
1050 Forest Hill Road,
Staten Island, New York 10314*

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Antigenic variation in three distinct determinants of an influenza type A haemagglutinin molecule

ALTHOUGH vaccines have been developed against many human viral pathogens, vaccination against influenza type A viruses has not been wholly successful. This is mainly due to the fact that the viral surface proteins, haemagglutinin and neuraminidase, are subject to antigenic evolution¹. Major changes in antigenicity (antigenic shifts) are attributed to (1) the occasional occurrence of genetic reassortment between antigenically dissimilar influenza viruses, or (2) the re-introduction of a previously prevalent influenza virus into the human population. In contrast, minor antigenic variation (antigenic drift) is thought to result from spontaneous point mutations during virus replication followed by the selection of mutants in a partially immune host population. Antigenic drift in the haemagglutinin molecule is epidemiologically of greater importance than that in the neuraminidase molecule, probably because immune mechanisms neutralise virus through interaction with the

Table 1 The frequency of antigenic variants present in cloned preparations of A/PR/8/34

Virus preparation	In absence of antibody	Virus titre ($\log_{10}$)					
		In presence of hybridoma antibody					
		B		C		L	
	(1)	(1)	(2)	(1)	(2)	(1)	(2)
Plaque E	7.46	1.35	(-6.11)	1.41	(-6.05)	0.94	(-6.52)
Plaque G	7.37	2.00	(-5.37)	2.20	(-5.17)	1.36	(-6.01)
Plaque H	6.99	2.25	(-4.74)	1.43	(-5.56)	0.75	(-6.23)
Plaque I	7.55	ND	ND	2.00	(-5.55)	1.19	(-6.36)
Plaque K	7.41	1.36	(-6.05)	ND	ND	ND	ND
Mean $\pm$ s.d.		(-5.57 $\pm$ 0.65)		(-5.57 $\pm$ 0.37)		(-6.26 $\pm$ 0.25)	

Influenza virus A/PR/8/34 (H0N1) was plaqued under agar at limit dilution on monolayers of MDCK cells. Five individual virus plaques were picked and the virus from each plaque was inoculated at 33-fold dilutions into the allantoic cavity of a 10-day-old embryonated hen's egg. After incubation at 35 °C for approximately 40 h, the allantoic fluid of one positive egg at the virus dilution end point was collected and stored frozen until used for infectivity titration in the AOS culture system in the absence or presence of hybridoma antibodies H2/6C4 (B), H2/4B3 (C) or H9/D3 (L). Briefly, dilutions of virus were preincubated for 1 h at room temperature in culture medium with or without antibody. A piece of allantois on shell was then added and samples were incubated for another 90 min at 37 °C. Following this infection period, the medium was removed and replaced by fresh virus-free medium that contained, in the case of titration in the presence of antibody, one-half of the antibody concentration present during infection. This was done to prevent any residual parental virus from infecting the AOS piece during the following incubation period. The AOS cultures were assayed for virus growth by haemagglutinin (HA) titration⁵ after 3 days incubation at 37 °C. The experimental system has the following characteristics. First, the concentration of ascitic fluid used did not result in nonspecific reduction of the virus titre. Second, the HA titration of the culture fluids after the 3-day incubation period provided an accurate parameter for infection and virus growth in that, at the titration end point, none of 24 HA-negative culture fluids showed evidence for the presence of infectious virus when inoculated into the allantoic cavity of embryonated eggs. The concentration of infectious virus (V_i) in the undiluted sample was calculated according to the Poisson formula: $V_i = -\ln P_i(0)/d_i$, where $P_i(0)$ equals the fraction of AOS cultures that are negative in the HA test at the virus dilution d_i . The titration was carried out at 10-fold virus dilution steps and 6–12 replicate AOS cultures were tested at each dilution. An estimate of V_i could usually be obtained from two consecutive virus dilution steps; in this case, the two values were averaged to give the value of V_i . (1) Infectious units of virus in 0.025 ml of allantoic fluid; (2) ratio of virus titre in the presence and absence of antibody. ND, not determined.

haemagglutinin molecule. It seems, therefore, that a prerequisite of antigenic drift is a high mutation rate in the antigenic structures of the haemagglutinin molecule. In the present study, the frequency of antigenic mutant viruses present in cloned virus preparations was determined by means of monoclonal antibodies. It was found that antigenic changes in individual determinants of the haemagglutinin molecule of A/PR/8/34 (H0N1) occurred with an average frequency of 10^{-6} per infectious dose of virus and that the three determinants described here mutated independently of each other. The epidemiological significance of these observations is discussed.

The frequency of antigenic mutant viruses present in cloned preparations of egg-grown influenza virus A/PR/8/34 was determined by parallel titration of the virus in the allantois on a shell culture system (AOS)^{2,3} in the presence or absence of BALB/c ascitic fluid containing monoclonal anti-haemagglutinin antibodies⁴.

The titration of five plaque-cloned preparations of A/PR/8/34 influenza virus in the AOS system (Table 1) showed that none of the three monoclonal anti-PR8 antibodies was able to neutralise the infectivity of the various virus inocula completely; on average, one out of $10^{5.5}$ to $10^{6.2}$ infectious units of the virus inocula were not neutralised. To exclude the possibility that virus growth represented parental virus and not antigenic variants, putative variant cultures were inoculated into the allantoic cavity of embryonated eggs and then analysed antigenically in an indirect radioimmunoassay (RIA)⁶. As shown in Table 2, viruses grown in the presence of antibody, in contrast to the parental virus inoculum, showed a greatly decreased capacity to bind the antibody used in the titration. These viruses, therefore, represent antigenic variants of the

parental PR8 virus. As the titre of variant viruses was the same whether antibody was present during the infection period only or during both the infection and incubation periods, it is concluded that the antigenic variants pre-existed in the virus preparations used for infection and must, therefore, have arisen from one infectious unit of the plaque-cloned parental virus during replication in the allantoic cavity of the embryonated egg. Assuming further that replication *in ovo* took place in the absence of any selective pressure for or against antigenic variants, the average frequency of the variants in the different allantoic fluids should provide a reliable estimate of their rate of occurrence.

Antigenic variations in the determinants delineated by the monoclonal antibodies B, C and L (that is, epitope B, C and L of the parental haemagglutinin) occurred with an average frequency of $10^{-5.5}$, $10^{-5.5}$ and $10^{-6.2}$ per infectious dose, respectively. This high frequency, in conjunction with a previous comparison of peptide maps and amino acid composition of parental and variant haemagglutinins⁷, is compatible with the idea that antigenic variants selected with monoclonal antibodies generally represent single point mutants of the parental virus.

The present study further shows that the three haemagglutinin epitopes B, C and L mutate independently of each other. For instance, mutations in epitope B (B variants) did not detectably change epitopes C or L; the reverse was also true (Table 2). Thus, the three epitopes do not seem to have a mutable contact area in common, that is, they may be defined on an operational basis as non-overlapping structures of the antigenic area of the haemagglutinin molecule. To test this concept further, virus was titrated in the presence of two monoclonal antibodies (Table 3). Given that the frequencies of antigenic variants in epitopes B and C are $10^{-5.06}$ and $10^{-5.11}$ per infectious dose of PR8, respectively, and assuming that these variants represent single point mutants of the parental virus, a variant with a mutation in both the B and C epitopes (B-C variant) should theoretically occur at a frequency equal to the product of the individual mutation rates, that is, $10^{-10.17}$. The fact that no B-C variant (frequency less than $10^{-9.13}$ per infectious dose) was observed in this experiment, supports the concept of the independent mutability of these epitopes. In this context, it should be noted that double variants are easily made through sequential selection (unpublished results) or through the use of hybridoma antibodies directed against two partially overlapping epitopes (unpublished results).

Current studies indicate that the size of the antigenic area of the haemagglutinin of PR8 corresponds to a minimum of four non-overlapping epitopes (in preparation). Thus, assuming that the normal antiviral immune response *in vivo* comprises antibodies directed against all parts of the antigenic area, only

Table 2 Antigenic analysis of virus isolated in the presence of antibody

Antibody present during titration of virus in AOS cultures	No. of AOS cultures analysed	Reaction of virus in RIA with antibody		
		B	C	L
B	13	-	+	+
	5	+/-	+	+
C	42	+	-	+
	1	+	+/-	+
L	2	+/-	-	+
	18	+	+	-
	3	+/-	+	-

Individual AOS cultures which produced virus in the presence of antibody B, C or L (see Table 1) were inoculated into the allantoic cavity of embryonated eggs. The egg-grown virus was then analysed antigenically by means of the RIA as previously described⁶. Briefly, replicate samples of 20 haemagglutinating units of the egg-grown virus derived from individual AOS cultures were bound to wells of polyvinyl plastic plates. The various viral immunoadsorbents were then incubated with constant quantities of hybridoma antibody B, C or L. The amount of hybridoma antibody bound to each immunoadsorbent was determined by means of radioiodinated rabbit anti-mouse F(ab')₂ antibody. The symbols (+, +/-, -) relate to the percentage of c.p.m. observed in the reaction of the given antibody with the test virus compared with its reaction with the parental virus inoculum: + $\geq$ 60%; +/- < 60%, > 15%; - $\leq$ 15%.

Table 3 Effect of the simultaneous presence of two monoclonal antibodies on the selection of antigenic variants of PR/8/34

	Antibody used for selection		
	B	C	B + C
No. of infectious doses analysed	10 ^{5.96}	10 ^{5.96}	10 ^{9.13}
No. of variants observed	8	7	0
Frequency of variants	10 ^{-5.06}	10 ^{-5.11}	<10 ^{-9.13}

PR8 virus was titrated in the AOS culture system (see Table 1 legend) with hybridoma antibodies B and C being present individually or simultaneously. Number and frequency of antigenic variants of PR8 were determined as given in the Table 1 legend.

quadruple mutants might actually have a good chance of escaping neutralisation *in vivo*. The estimated frequency of 10⁻²⁴ for such a virus variant would probably be too low to account for the regular occurrence of antigenic drift in human influenza type A viruses. However, the fact that antigenic drift does occur in nature may mean that: (1) antibody against an individual epitope does not totally neutralise *in vivo*; (2) the frequency of variation is higher during replication of an influenza virus in the human respiratory tract than *in ovo*; (3) humans possess, on average, a more restricted anti-influenza antibody repertoire than do mice (from which the hybridoma antibodies originated); (4) certain individuals possess an antibody repertoire restricted to one or two non-overlapping epitopes, a phenomenon which would allow the virus to undergo antigenic drift in a sequential manner.

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J. W. YEWDELL*

R. G. WEBSTER†

W. U. GERHARD*

* The Wistar Institute of Anatomy and Biology,
36th Street at Spruce,
Philadelphia, Pennsylvania 19104

† St Jude Children's Research Hospital,
Memphis, Tennessee

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Dog red blood cells exhibit a Ca-stimulated increase in K permeability in the absence of (Na,K)ATPase activity

ERYTHROCYTES of man and certain other animals exhibit a selective increase in K permeability as a result of the action of micromolar concentrations of Ca (or Pb) at the inner surface of the membrane^{1–4}. Based on the fact that inhibitors of (Na,K)ATPase, such as ouabain, furosemide, oligomycin and tetraethylammonium ion, also inhibit the Ca-dependent K permeability increase, Blum and Hoffman^{5,6} have postulated that an altered form of the (Na,K)ATPase might be involved in increasing the K permeability. Dog red cells differ from those of man and most other mammalian species in that they have a high Na content and do not have any detectable (Na,K)ATPase^{7–9} or

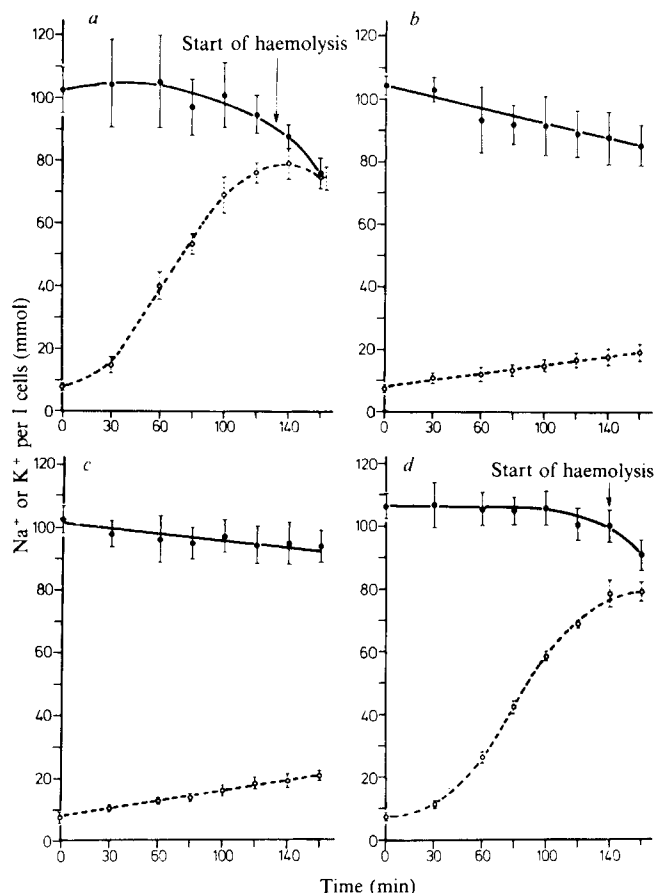


Fig. 1 Na and K content of RBC plotted as a function of time after exposure to various media. Freshly drawn blood from beagle dogs (with heparin as anticoagulant) was washed with ice-cold 113 mM MgCl₂ and white cells were removed. The cells were washed two more times and then were added (at a final average haematocrit of about 9%) to media at 37°C containing 100 mM KCl, 50 mM NaCl and other components as indicated below. The haematocrit was taken at the beginning of the experiment and 0.5-ml samples were pipetted at various times into 9 ml ice-cold 113 mM MgCl₂. After centrifugation at 4°C, the supernatant was removed and cells were washed once more with MgCl₂. The cell pellet was haemolysed in 3.8 ml double distilled water and 0.2 ml detergent (Netzmittellösung-Eppendorf). After centrifugation the concentrations of Na and K in the haemolysates were determined with an Eppendorf flame photometer, and the Na and K contents of the cells were expressed per litre of initial cell volume, as determined from the haematocrit at the start of the experiment. ●—●, Na content; ○—○, K content. Bars indicate the s.d. of four experiments. Conditions were as follows:

Figure	CaCl ₂ (mM)	EDTA (mM)	Adenosine (mM)	Iodoacetic acid (mM)
a	0.5	0	11	2.8
b	0.5	0	11	0
c	0	1	11	2.8
d	0.5	0	0	2.8

ouabain-sensitive Na pump activity^{9,10}. We report here results demonstrating that the Ca-stimulated K permeability increase can occur in these red cells which lack any demonstrable (Na,K)ATPase, thereby indicating that (Na,K)ATPase is not required for the Ca-induced increase in K permeability.

The Ca-dependent increase in K permeability has been observed in metabolically depleted red blood cells (RBC)^{5,11,12}, propranolol treated cells^{13,14}, cells treated with the Ca ionophore A23187^{15,16} and erythrocyte ghosts^{17–19}. The function of this selective permeation system in RBC is obscure, but it could be involved in the regulation of the volume and potassium content of the cell. In addition, this system provides a possible

model for other Ca-dependent K-gating processes which may have important physiological roles in other cells²⁰⁻²⁴.

Support for the concept that (Na,K)ATPase is involved in the selective K permeability increase has come from observations that the system exhibits selective interactions with alkali metal ions at both sides of the membrane^{5,6,18,19} and that transmembrane effects occur^{6,19}, suggesting that a carrier-mediated system is involved in the permeability increase. However, an alternative explanation has been proposed for the ouabain effects²⁵, and Jenkins and Lew²⁶ have found that some animal RBC having (Na,K)ATPase do not exhibit a Ca-stimulated increase in K permeability. Sheep reticulocytes, which have a much higher (Na,K)ATPase activity than do mature sheep RBC, still do not manifest any Ca-stimulated K flux²⁷. Nevertheless, all cells so far studied which do exhibit the K permeability increase have (Na,K)ATPase. In an attempt to investigate the possible involvement of (Na,K)ATPase, therefore, we asked whether or not the Ca-stimulated K permeability increase could be observed in dog RBC, which have no detectable (Na,K)ATPase.

The conditions chosen for these experiments were similar to those originally described by Gardos¹¹ for human RBC (see Fig. 1 legend). Metabolic depletion was achieved by supplying the cells with adenosine in conditions where glycolytic metabolism was blocked by iodoacetic acid²⁵. To avoid complications due to possible K-K exchange processes, net K transport was observed by monitoring the K content of the cells with time. The K selectivity of the permeability change was checked by simultaneously determining the Na content of the cells. In the case of human RBC, which have a high K concentration, the selective K permeability increase has normally been observed by measuring K loss into a low K medium^{2,5,6,11-17,19}. As dog RBC have a low internal K concentration, we chose to measure K influx into the cells from a high K medium.

As shown in Fig. 1a, in conditions of metabolic depletion and in the presence of Ca, dog RBC exhibit a large influx of K. At times up to 100 min, when the K influx is fully manifest, there is little change in Na content, demonstrating that the permeability increase is selective for K. At later times when, because of cell swelling resulting from the influx of K, the cells begin to haemolyse, some Na loss is observed. If glycolysis is not inhibited by iodoacetic acid there is no selective increase in K permeability (Fig. 1b). Figure 1c shows that Ca is required for the permeability increase, as no effect is observed in the media to which the Ca chelating agent, EDTA, was added instead of Ca. Adenosine is not required for the K permeability increase (Fig. 1d), although the effect appears to develop somewhat more slowly without adenosine. This figure once again demonstrates the selectivity of the permeability change, as no detectable efflux of Na is observed during the period of maximal K influx. The absence of an adenosine requirement^{28,29} may reflect differences in the metabolism of dog RBC compared with human RBC.

Other investigators have reported increases^{10,30} or decreases^{10,31} in K permeability after metabolic depletion, but no Ca dependence was observed¹⁰. The discrepancies between these earlier results and the present findings may be partly due to the different experimental conditions used and to the fact that in the present work, net K fluxes were measured rather than ⁴²K exchange. A recent report³² that propranolol and Ca cause an increased specific net K influx into dog RBC suspended in high K media is consistent with these findings.

The selective increase in K permeability observed in these experiments appears to be distinct from the changes in K permeability observed in other circumstances in dog RBC. External ATP has been shown to increase K permeability^{33,34}, but this is accompanied by a Na permeability increase, in contrast to the results shown in Fig. 1a and d. When dog RBC are swollen, a selective increase in K permeability results^{31,35}. This does not explain the present data, however, as no permeability change occurred in the absence of iodoacetate (Fig. 1b) or Ca (Fig. 1c). Furthermore, metabolic depletion decreases the effect of cell volume on K permeability³¹, but facilitates the

Ca-stimulated K permeability increase (compare Fig. 1a and b). Nevertheless, possible effects of cell swelling on the permeability change were experimentally tested (Fig. 2a,b) by adding 100 mM mannitol to the medium to decrease the initial cell volume. This caused a slight increase in Na permeability, as expected, demonstrating that the cells were well below the volume at which changes in K permeability occur³⁵. The magnitude of the Ca-induced K influx was slightly reduced by mannitol. This may be partly due to the increase in cell K concentration caused by cell shrinkage, which decreases the driving force for net K influx. It may also indicate that the swelling of the cells beyond their normal volume, which increases Ca influx³⁶, enhances the Ca-stimulated K influx. Regardless of the explanation for the slight decrease in K influx, the fact that Ca does increase the K flux in shrunken cells demonstrates that the triggering of the effect is independent of cell volume.

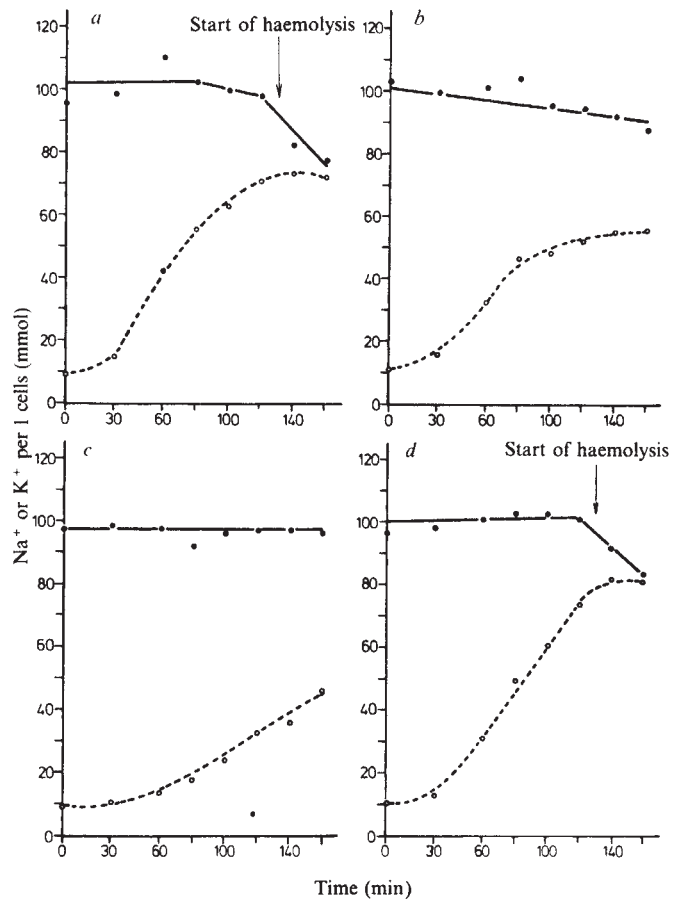


Fig. 2 Effects of hypertonicity and inhibitors on the Ca-stimulated K influx into dog RBC. Conditions were as described for Fig. 1a. a, No additions; b, 100 mM mannitol added; c, 20 $\mu\text{g ml}^{-1}$ oligomycin added; d, 0.15 mM ouabain added. The pH in all experiments was 7.2–7.5 at 37°C. As in Fig. 1, the Na content (●—●) or K content (○—○) of the cells per litre of initial cell volume are plotted against time.

Oligomycin, which inhibits the Ca-stimulated K flux in human RBC by more than 80% at a concentration of 10 $\mu\text{g ml}^{-1}$ or less^{5,6}, had a smaller inhibitory effect in dog RBC (Fig. 2c). Ouabain had no effect (Fig. 2d), even at the very high concentration of 0.15 mM, consistent with the concept that the permeability increase does not require the participation of a Na,K pump. Quinidine, which inhibits Ca influx³⁷, and which also has a direct inhibitory effect on the Ca-induced K flux in human RBC²⁴ and sheep fetal RBC²⁷, caused substantial inhibition of the K flux at 1 mM, but this was partly obscured by a generalised increase in cation permeability and haemolysis caused by quinidine.

Dog erythrocytes provide a system in which the effects of Ca on K permeability can be observed and studied without the complications introduced by the Na,K pump. The results presented in Figs 1 and 2 provide evidence that Ca induces a selective increase in K permeability, similar to that which occurs in other RBC, in dog RBC despite their apparent lack of (Na,K)ATPase. Therefore, it seems that (Na,K)ATPase is not a necessary element in the selective K permeability increase, although it could have a subsidiary role in cells which have this enzyme.

In support of this study Parker *et al.* have found that if dog RBC are loaded with potassium by incubation in a high K medium with 1 mM ATP^{33,34}, and are then suspended in a low K medium with iodoacetic acid and adenosine at 37°C, addition of 5 mM calcium causes a pronounced selective loss of K from the cells (over 50% in 3 h), and a concomitant shrinkage in cell volume. These data also provide evidence that the selective increase in K permeability is not primarily related to cell volume changes, as it can occur in conditions where an increase in K permeability causes cell swelling (this study) or cell shrinkage (J. C. Parker, unpublished results).

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HANS-W. RICHHARDT
GUNTER FRED FUHRMANN

Institut für Pharmakologie und Toxikologie,
Philipps-Universität Marburg,
D 3550 Marburg, FRG

PHILIP A. KNAUF

Research Institute,
The Hospital for Sick Children,
Toronto, Ontario, Canada M5G 1X8

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Interactions of lysophospholipids and mast cells

PHOSPHOLIPID and protein are the major components of biological membranes, and much is known about their organisation in various cell membranes^{1,2}. An important question concerns the existence and significance of specific protein-phospholipid interactions in membranes. Among the methods used to elucidate these interactions is the study of the regulatory function of specific phospholipids in intact cells. Phosphatidylserine (PS) enhances the secretory response of the mast cell³ in a highly specific manner. Other diacyl phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol, phosphatidylinositol and phosphatidic acid, are ineffective³⁻⁵ and several N-substituted derivatives of PS are competitive inhibitors of the effect of PS on mast cells⁶.

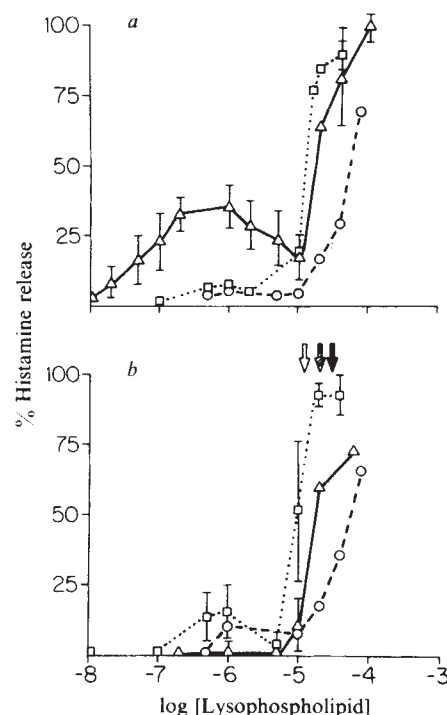


Fig. 1 Effect of lysophospholipids on histamine release from rat peritoneal mast cells in the presence (a) and absence (b) of Con A. Mast cells obtained from the peritoneal cavity of adult male rats were purified to greater than 95% homogeneity by centrifugation through albumin¹⁹. The cells (2×10^5) were suspended in 0.75 ml of BSS and incubated for 2 min at 37°C with various concentrations of lysophospholipid. Ca^{2+} (0.68 μmol) and Con A (100 μg where indicated) were then added in 0.25 ml of BSS and the cells incubated for an additional 15 min at 37°C. Histamine in the supernatant and in perchloric acid extracts of cell pellets was determined by a modification of the *o*-phthalaldehyde, fluorometric method²⁰ after centrifugation of the cells at 220g for 5 min. Histamine release is expressed as a per cent of total cell histamine after correcting for spontaneous release in controls in which lysophospholipid was omitted from the original incubation medium. Spontaneous release never exceeded 8% of total cell histamine and was not significantly affected by the presence of Con A. The concentrations of lyso-PE (○---○), lyso-PC (□---□) and lyso-PS (△---△) shown are the concentration of lysophospholipid in the final 15-min incubation. Each datum point represents the average of results from at least two experiments with duplicate determinations in each experiment. The bars designate s.e.m. in those experiments carried out three or more times. The arrows in the lower panel indicate the respective log CMCs determined for lyso-PC (open, -4.9), lyso-PS (hatched, -4.75), and lyso-PE (filled, -4.5). The CMCs were determined in BSS with the fluorescent probe, *N*-phenyl-1-naphthylamine²¹.

Furthermore, PS selectively enhances the response of cells to IgE-dependent secretagogues^{7,8} without increasing the response of the cells to IgE-independent agents such as polymyxin B⁹, chymotrypsin³, compound 48/80 (refs 3, 9), or A23187 (D. L. and T. W. M., unpublished). As histamine secretion involves a major alteration in the organisation of membrane components¹⁰, the effect of PS on secretion makes it possible to study a complex membrane event with an established phospholipid specificity. Previously, we have used PS of various fatty acid compositions¹¹: dimyristoyl PS, dipalmitoyl PS, and distearoyl PS. We now provide evidence that lysophosphatidylserine (lyso-PS) generated from bovine brain PS by the action of phospholipase A₂ activates mast cell secretion induced by concanavalin A (Con A) at 1/30th to 1/50th the concentration of PS required for an equivalent response. Although some characteristics of the activation of mast cell secretion by lyso-PS resemble those observed with PS, its mode of interaction with the cell may be different; PS acts on mast cells in a micellar state¹², but lyso-PS acts to potentiate secretion well below its critical micelle concentration (CMC).

PS was isolated from bovine brain¹³, and PE and PC were isolated from bovine erythrocytes and egg yolks, respectively, by silicic acid column chromatography¹⁴. The three phospholipids migrated as single spots on silica gel H plates developed in diisobutylketone/acetic acid/water (40:30:7, v/v). The lyso derivatives were generated from the purified phospholipids by treatment with *Crotalus adamanteus* phospholipase A₂ (Sigma); lysophospholipids were purified by silicic acid column chromatography and migrated as single spots on thin-layer chromatography in the solvent system described above. Acetimidyl-lyso-PS was synthesised from lyso-PS and methyl acetimidate (Pierce). The reaction used a 10-fold molar excess of methyl acetimidate which was added as the dry powder to 20 μ mol of lyso-PS dissolved in 2 ml of anhydrous CHCl₃ and 0.1 ml of triethylamine. After 30 min at room temperature, another 10-fold excess of reagent was added and the reaction was continued for 30 min. After extensive extraction with an equal volume of 0.001 M HCl and water, the CHCl₃ phase was evaporated to dryness *in vacuo* and dissolved in anhydrous CHCl₃. More than 95% of the free amino groups of lyso-PS were amidinated in these conditions as measured by reaction with trinitrobenzenesulphonic acid¹⁵. PS was amidinated with methyl acetimidate by a similar procedure.

Table 1 Characteristics of Con A-induced histamine secretion potentiated by PS and lyso-PS

Preincubation	Incubation	% Histamine release
	Ca ²⁺ (6)	6.6 $\pm$ 0.8
	Lyso-PS + Con A + Ca ²⁺ (13)	39.0 $\pm$ 2.7
	PS + Con A + Ca ²⁺ (3)	31.6 $\pm$ 3.0
	Ac-lyso-PS + Con A + Ca ²⁺ (5)	6.4 $\pm$ 1.2
	Ac-PS + Con A + Ca ²⁺ (3)	6.9 $\pm$ 1.4
Antimycin A	Lyso-PS + Con A + Ca ²⁺ (6)	6.3 $\pm$ 0.9
Lyso-PS	Con A + Ca ²⁺ (5)	35.1 $\pm$ 6.1
Lyso-PS	Con A (3)	9.6 $\pm$ 0.6
Lyso-PS	Ca ²⁺ (5)	7.6 $\pm$ 0.8

Aliquots of peritoneal cells containing 2×10^5 mast cells obtained from adult male rats were incubated in 1.0 ml of balanced salt solution (BSS: 4 mM Na₂HPO₄, 2.7 mM KH₂PO₄, 150 mM NaCl, 2.7 mM KCl, pH 7.2) with the indicated combinations of lyso-PS (0.2 μ M), Con A (100 μ g ml⁻¹), Ca²⁺ (0.68 mM), acetimidyl-lyso-PS (Ac-lyso-PS, 0.2 μ M), PS (50 μ M), or acetimidyl-PS (Ac-PS, 50 μ M) for 15 min at 37°C. Where indicated, the cells were preincubated at room temperature for 10 min with antimycin A (0.2 μ M) or lyso-PS (0.2 μ M) and washed before a second incubation at 37°C for 15 min. Histamine release is expressed as a mean per cent of total cell histamine ($\pm$ s.e.m.) after direct assay of supernatants and perchloric acid extracts of cell pellets as detailed in Fig. 1. Duplicate determinations were made in each experiment; the number of individual experiments is shown in parentheses.

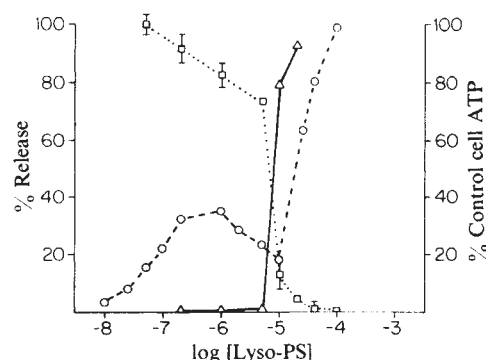


Fig. 2 Effect of lyso-PS on rat peritoneal mast cell ATP levels and release of histamine and lactate dehydrogenase (LDH). Mast cells were obtained from the peritoneal cavity of adult male rats and purified as in Fig. 1. The cells (4×10^5) were incubated with Con A (100 μ g ml⁻¹), Ca²⁺ (0.68 mM), and various concentrations of lyso-PS in a final volume of 1.0 ml of BSS according to the procedure detailed in Fig. 1. After centrifugation of the cells for 5 min at 220g, cell ATP levels²² and pellet and supernatant levels of LDH²³ were measured. LDH release (Δ — Δ) is expressed as the per cent of total cell LDH, and cell ATP ($\square$ — $\square$) as the per cent of control cell ATP. Each datum point represents the average of at least two experiments with duplicate determinations in each experiment. Bars designate s.e.m. in experiments carried out three or more times. The histamine release curve ($\circ$ — $\circ$) was that determined in Fig. 1 and is included here for comparison. Virtually identical ATP and LDH release curves were obtained in the absence of Con A.

The ability of lyso-PS, lyso-PE, and lyso-PC to activate Con A-induced histamine secretion was tested; the results are presented in Fig. 1. Treatment of mast cells with any of the three lysophospholipids above 10 μ M resulted in a substantial histamine release that was independent of the presence of Con A. Of the three, only lyso-PS was capable of eliciting Con A-dependent histamine release at concentrations below 10 μ M. Although it is apparent from Fig. 1 that cells exposed to 1 μ M lyso-PC release significant amounts of histamine, the comparatively low level of release is inhibited by the addition of Con A, rather than augmented, as is the case with lyso-PS.

Lysophospholipids are single-chain amphiphiles capable of forming detergent micelles in aqueous media when suspended at concentrations above their CMC¹⁶. We propose that the biphasic lyso-PS dose-response curve is the consequence of the differential action of lyso-PS on the mast cell above and below its CMC. Below the CMC, lyso-PS interacts non-cytotoxically with the mast cell and this interaction potentiates Con A-induced histamine secretion. At and above the CMC, detergent micelles lyse the cells and release histamine by a cytolytic mechanism. This proposal is supported by the observation that although the three lysophospholipids elicit substantial Con A-independent histamine release at and above their respective CMCs, only lyso-PS is capable of enhancing Con A-dependent release below its CMC.

The data in Fig. 2 support a non-cytotoxic mechanism of histamine release from mast cells treated with lyso-PS and Con A. No release of the cytoplasmic enzyme, lactate dehydrogenase (LDH), occurs when mast cells are treated with concentrations of lyso-PS below the CMC, and cellular ATP levels are not decreased below those consistent with normal cell ATP utilisation until the CMC of lyso-PS is attained. It is evident from Fig. 2 that the midpoint of the secondary ascending limb of the histamine release curve occurs in the presence of marked loss of ATP and release of LDH and is close to the CMC. Thus, these events probably result from lytic action of lyso-PS micelles.

Table 1 shows the results of investigation of several additional characteristics of histamine secretion induced by lyso-PS and Con A. When mast cells are pretreated with 0.2 μ M lyso-PS in

the absence of Con A and Ca^{2+} , secretion after subsequent washing and the addition of Con A requires Ca^{2+} . This experiment demonstrates that lyso-PS is capable of interacting effectively with the cell in the absence of Con A and Ca^{2+} , but that all three components are required for secretion. Con A-induced secretion activated by lyso-PS is inhibited when mast cell ATP levels are depressed by treatment with antimycin A. The Ca^{2+} and ATP dependence provide additional evidence against the possibility of cytotoxicity as an explanation for the effect of lyso-PS on Con A-induced secretion.

The finding that lyso-PC and lyso-PE are incapable of potentiating histamine secretion indicates head-group specificity in the action of lyso-PS similar to that previously documented for PS⁶. (Our negative results with lyso-PC do not agree with the report of Sydbom and Uvnäs¹⁷, who presented data indicative of a potentiating effect of 3–13 μM lyso-PC on antigen-induced histamine release. The difference in primary secretagogue—antigen in their experiments and Con A in ours—may be the basis for the different results. However, although maximum non-cytotoxic lyso-PS-activated release occurs at 1/100th the cytotoxic concentration in our experiments, maximum lyso-PC potentiation was observed by Sydbom and Uvnäs at one-third to one-half of its cytotoxic concentration.) The structural requirement of the lyso-PS head group was further examined with an *N*-substituted lyso-PS derivative. Methyl acetimidate was used to convert the primary amino group of PS and lyso-PS to an amidine which, like the original primary amine, is protonated at physiological pH¹⁸. The finding that this minor structural modification completely abolishes activity indicates that the stringent structural requirement for an effective PS head group is retained in the action of the lyso derivative.

The concentration of lyso-PS required to achieve 50% of the maximum response is 0.065 μM (Fig. 1), whereas 3–5 μM PS is required for an equivalent response¹². This finding and the similarities in the general requirements for the effects of lyso-PS and PS on Con A-induced secretion suggest that potentiation by PS may involve its conversion to lyso-PS by a mast cell phospholipase A. Alternatively, it may be that both PS and lyso-PS are capable of potentiating secretion by virtue of the unique chemical structure of their polar head groups, and the greater potency of lyso-PS is a reflection of its ability to perform this function more efficiently. Direct experiments to test the hypothesis that the potentiating effect of PS on mast cell secretion requires conversion to lyso-PS are in progress.

It must also be considered that the potentiating effect attributed to PS may be the consequence of lyso-PS contamination. An upper limit for the proportion of lyso-PS detectable in our PS preparations isolated from bovine brain and used to determine the comparative dose-response curves is 2.5%. This amount of lyso-PS impurity is nearly sufficient to account for the activity of PS. Further studies of the efficacy of lyso-PS in the presence of excess PS and of PS with lower levels of lyso-PS contamination are required to resolve this question.

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THOMAS W. MARTIN
DAVID LAGUNOFF

Department of Pathology,
University of Washington,
Seattle, Washington 98195

Received 5 January; accepted 20 March 1979.

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Endorphins are stored in biologically active and inactive forms: isolation of α -*N*-acetyl peptides

SINCE the C-fragment of lipotropin (residues 61–91, β -endorphin) was found to occur naturally in the pituitary^{1,2} and brain^{3,4}, the opiate-like properties of this peptide and its possible involvement in regulatory mechanisms in the central nervous system have been intensively studied. The C'-fragment of lipotropin (residues 61–87) also occurs in the pituitary¹; it is a much less active opiate and has been little studied. We report here further experiments which reveal that in porcine pituitary the C- and C'-fragments are accompanied by substantial quantities of the corresponding α -*N*-acetyl peptides. The acetyl forms are inactive in opiate binding assays and in tests for analgesic properties but they retain the full immunological potency of the parent peptides.

The acetyl peptides were isolated from whole porcine pituitaries (500 g) by a modification of a procedure described for the preparation of C-fragment⁴. Homogenisation was in acid acetone to which [¹²⁵I]C-fragment (~1 μg , 10⁵ c.p.m.) was added, allowing estimation of recovery at each stage of the isolation. After precipitation by saturation with acetone and separation from large polypeptides by salt precipitation, the extracted peptides were desalted on Sephadex G-25 in 1 M acetic acid. An additional step was introduced before ion-exchange chromatography: material sparingly soluble at neutral pH was separated by centrifugation, which removed most of the corticotropin and left the C- and C'-fragments in solution. This aided the subsequent resolution of corticotropin from *N*-acetyl C-fragment and led to the recognition of the acetyl peptides. Chromatography of the peptide mixture on CM-Sephadex C-25

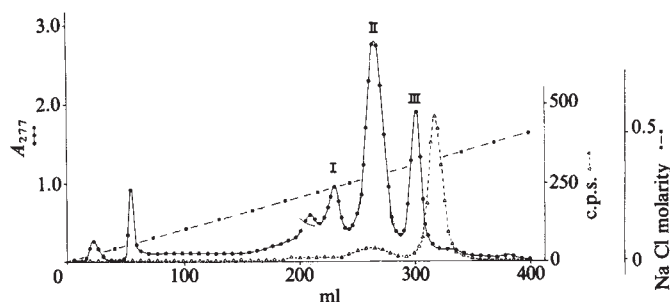


Fig. 1 Chromatography of basic peptides extracted from porcine pituitary. The peptides were eluted from a column (70 × 0.9 cm) of SP-Sephadex C25 in 0.02 M sodium phosphate at pH 7.0 with a linear gradient from 0 to 0.5 M sodium chloride. The mixer volume was 200 ml and the fraction size 3.2 ml. *N*-Acetyl C-fragment (I), corticotropin (II) and C-fragment (III) emerged in the positions indicated; [¹²⁵I]C-fragment (Δ) was eluted after the unlabelled peptide.

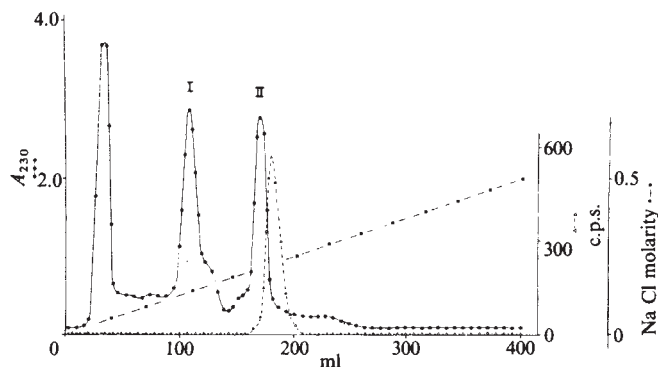


Fig. 2 Chromatography of a neutral peptide fraction extracted from porcine pituitary. The conditions used were those of Fig. 1. *N*-Acetyl C'-fragment (I) and C'-fragment (II) emerged in the positions indicated; [125 I]C'-fragment (Δ) was eluted after the unlabelled peptide.

provided three fractions: acidic peptides not retained at pH 4.5, relatively neutral peptides eluted in 0.2 M sodium phosphate at pH 6.8, and a group of basic peptides eluted in 1 M sodium phosphate at pH 6.8. Further chromatography of the basic peptides revealed a new component (peak I, Fig. 1), which after gel filtration on Sephadex G-50 in 50% acetic acid gave the homogeneous peptide, α -*N*-acetyl C-fragment.

The new peptide was less basic than the C-fragment but seemed to possess the same amino acid composition (Table 1). Digestion with trypsin and aminopeptidase M, however, failed to release the N-terminal tyrosine or the adjacent glycine residue, although the remaining amino acids corresponded to those obtained by enzymatic digestion of the C-fragment. This indicated that the N-terminal tyrosine residue in the C-fragment variant was modified by acylation.

During the isolation of acetyl C-fragment we noted that the iodinated C-fragment chromatographed in a single position and that no radioactivity emerged with the acetyl peptide (Fig. 1). We concluded that *N*-acetyl C-fragment occurs as a natural substance in the pituitary gland and is not formed as an artefact of the isolation procedure.

Chromatography of the neutral fraction from CM-Sephadex C25 revealed a second new peptide (Fig. 2), with composition identical to that of the C'-fragment (Table 1), and enzymatic digestion again confirmed that the terminal tyrosine residue was modified. In a series of extractions from porcine pituitary the yield of this *N*-acetyl peptide ranged from 70 to 100% of that of the C'-fragment, whereas the yield of *N*-acetyl C-fragment was in the region of 30% of that of its NH_2 analogue.

Table 1 Amino acid composition of the C- and C'-fragments of lipotropin and their acetyl derivatives isolated from porcine pituitary

	<i>N</i> -acetyl C'-fragment	C'-fragment	<i>N</i> -acetyl C-fragment	C-fragment
Asp	1.9	2.1	2.3	2.0
Thr	3.0	2.9	2.7	2.9
Ser	2.0	1.9	2.0	1.9
Glu	2.1	2.0	3.1	3.0
Pro	0.9	1.0	1.1	1.1
Gly	2.1	2.1	2.8	3.0
Ala	1.9	2.0	2.3	1.9
Val	1.3	1.4	1.3	1.5
Met	0.9	1.0	0.3	0.9
Ile	0.4	0.4	0.4	0.5
Leu	1.9	2.1	2.1	1.9
Tyr	1.0	1.0	0.9	0.9
Phe	1.8	2.0	1.8	1.9
Lys	3.2	3.1	5.3	5.1
His	0.9	1.1	0.9	0.9

Hydrolysis was carried out in 6 M hydrochloric acid for 16 h at 110 °C and analysis was on a Beckman 120C amino acid analyser. The values are expressed to the nearest one-tenth of one residue.

The acyl substituent in the two peptides was identified as an acetyl group by mass spectrometric analysis. The *N*-acetyl tyrosine was liberated from each peptide by digestion with chymotrypsin and the acyl amino acid was isolated in >90% yield by passage through a column of Dowex 50 $\times$ 2 (H^+ form). Electron impact mass spectra of authentic *N*-acetyl tyrosine and of the *N*-acetyl tyrosine from the isolated peptides were obtained on a Varian 311A instrument at 150 °C and 70 eV ionisation energy. Low resolution fragmentation (Fig. 3) and accurate mass measurements of the major ions at high resolution were identical. The values for the latter agree closely with those calculated from the expected empirical formulae (Table 2).

Table 2 Mass measurements of the ions in the spectra of the N-terminal residue of *N*-acetyl C-fragment and of authentic *N*-acetyl tyrosine

Nominal mass	Empirical formula	Calculated mass	Measured mass	Error
223	$\text{C}_{11}\text{H}_{13}\text{O}_4\text{N}$	223.08545	223.09009	+0.00464
164	$\text{C}_9\text{H}_8\text{O}_3$	164.04734	164.04364	-0.00370
136	$\text{C}_8\text{H}_{10}\text{ON}$	136.07624	136.07059	-0.00565
135	$\text{C}_8\text{H}_9\text{ON}$	135.06841	135.06758	-0.00083
119	$\text{C}_8\text{H}_7\text{O}$	119.04969	119.04795	-0.00174
107	$\text{C}_7\text{H}_7\text{O}$	107.04969	107.04867	-0.00102
91	C_7H_7	91.05477	91.05305	-0.00172

The *N*-acetyl endorphins do not possess the potent opiate properties of the parent peptides. As expected, in displacing ^3H -naloxone from brain synaptosomes⁵, no specific binding properties were observed for the acetyl forms of the C- and C'-fragments; the I_{50} values were $>10^{-5}$ M. In this context, it has been demonstrated that the affinity of enkephalin (lipotropin residues 61–65) for brain opiate receptors is essentially eliminated by acylation of the terminal amino group^{3,6,7}. In agreement with the *in vitro* results, *N*-acetyl C-fragment injected intracerebroventricularly did not produce analgesia in the tail-pinch test in the cat⁸ or in the rat tail flick assay⁹ at doses up to 20 times the minimum effective dose of C-fragment.

The ability of the C- and C'-fragments to react with antibodies directed against C-fragment is maintained in full by the acetyl peptides¹⁰ and results obtained by immunofluorescence or immunoassay reflect the presence of the C'-fragment and the acetyl derivatives in addition to the C-fragment. However, compared with C-fragment, the other peptides are essentially inactive as opiates. Current studies (ref. 11, S.Z. and D.G.S. unpublished results) have mapped the distribution of the active and inactive forms of the C- and C'-fragments in the pituitary and brain of the rat, cat and pig; the results demonstrate specific processing patterns which are characteristic for anatomically distinct areas.

The acetylation reaction does not take place extracellularly. When [125 I]C-fragment (1 μg , $\sim 10^5$ c.p.m.) was injected intracerebroventricularly in the cat, no formation of labelled acetyl derivative could be detected by chromatography of the cerebrospinal fluid over a 4-h period; similarly, no deacetylation of [125 I]-acetyl C-fragment was seen in the same conditions. The acetylation reaction seems to be a post-translational modification which occurs before or during the packaging of the peptides in the secretory granules. This seems to differ from the acetylation of certain proteins which takes place on the nascent polypeptide attached to the ribosome¹². The acetylation of C-fragment probably takes place concomitantly with the acetylation of α -melanotropin (α -MSH), the two peptides being derived from a common precursor¹³. As the acetyl group is important for the biological activity of α -MSH¹⁴ but eliminates the morphomimetic activity of C-fragment, the acetylation reaction may constitute a selective mechanism for the expression of activity associated with different regions of the 31,000-dalton prohormone. It is also possible, of course, that *N*-acetyl C-fragment possesses a biological activity unrelated to the

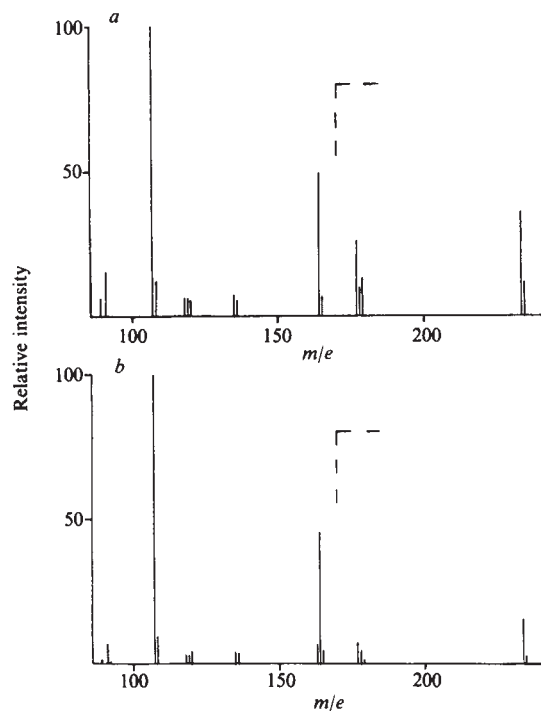


Fig. 3 Normalised partial mass spectra of the N-terminal amino acid of *N*-acetyl C-fragment (a) and authentic *N*-acetyl tyrosine (b).

opiate properties of the corresponding NH_2 -peptide and in this respect it could be relevant that a synthetic preparation of destyrosyl γ -endorphin has been reported to have a neuroleptic action¹⁵.

The potent opiate activity of C-fragment is severely impaired by the loss of its C-terminal tetrapeptide to form C'-fragment¹⁶ and the present results show that acetylation at the N-terminus also leads to inactivation. These reactions may be important for maintaining normal levels of opiate activity in the central nervous system, and malfunction of their regulation could lead to the abnormal concentrations of C-fragment considered to underlie certain neurological disorders¹⁷⁻¹⁹.

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D. G. SMYTH
D. E. MASSEY
S. ZAKARIAN

National Institute for Medical Research,
Mill Hill,
London NW7, UK

Department of Mass Spectrometry,
St. Bartholomew's Hospital,
London EC1, UK

M. D. A. FINNIE

Received 12 January; accepted 6 April 1979.

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An intermediate type of muscle fibre in *Xenopus laevis*

AMPHIBIAN skeletal muscle is generally regarded as being composed of two distinct fibre types, twitch fibres and slow fibres, and various morphological and functional criteria have been used to classify fibres into one of these two categories (for review see ref. 1). However, the results of several investigations on frog and toad, including *Xenopus*, muscles suggest that this is an oversimplification²⁻⁵. Smith and Ovalle⁶ demonstrated five classes of fibres in hind limb muscles of *Xenopus laevis* using various ultrastructural and histochemical criteria. The mechanical performances of fibres of two of these classes (fast, fatigue-resistant twitch fibres (type 2) and slow fibres (type 5), respectively) have recently been described in detail⁷. In the study reported here fibres with a characteristic microscopic appearance have been isolated from a hind limb muscle of *Xenopus laevis* and it is shown that they have functional properties which are intermediate between those of twitch and slow fibres. The fine structure of these fibres conforms to the description of Smith and Ovalle for the 'type 4' fibre, a fibre type which also can morphologically be said to represent an intermediary type.

The iliofibularis muscle of medium-sized *Xenopus laevis* females was used for all the experiments. Single fibres were dissected from the central zone of the muscle, in the immediate vicinity of the nerve entrance, using dark-field illumination and $\times 50$ -100 magnification. In these conditions differences in light-scattering properties of fibres are clearly seen, especially in well-fed animals, and can be used as a criterion for identifying fibres of various functional types². Transparent fibres with traces of light-scattering particle were selected. Electrical stimulation with a fine pipette was performed during late stages of the dissection. Completely transparent fibres responded with slow, local contractions; almost transparent fibres gave slow, all-or-none twitches or relatively brisk, local contractions.

The isolated fibres were transferred to a recording chamber with facilities for transverse electrical stimulation, quick solution changes and tension recording in isometric and isotonic conditions⁷. All experiments were carried out at $20 \pm 0.2^\circ \text{C}$. The Ringer solution used contained (mM): NaCl 115, KCl 2.5, CaCl_2 1.8, Na phosphate buffer 3.0; at pH 7.2. After completion of mechanical experiments several fibres were transferred to another trough, in which they were held at fixed length. The localisation of cholinesterase activity was studied in four fibres (without previous fixation) using the method of Karnovsky and Roots⁸; two other fibres were fixed in 4% glutaraldehyde in Ringer solution, postfixed in 2% buffered OsO_4 , dehydrated and embedded in Epon. Thin sections were cut with diamond knives, mounted on formvar-coated grids, stained with uranyl acetate and lead citrate, and examined in a Zeiss EM 9 electron microscope. One twitch fibre (type 1) and one slow fibre were also processed for electron microscopy and served as controls.

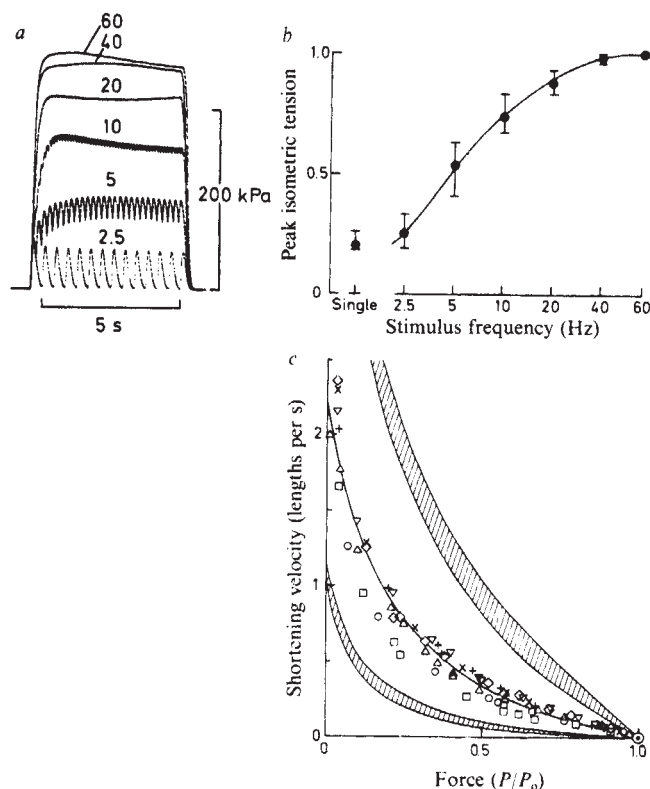


Fig. 1 *a*, Mechanical responses of a fibre to repetitive 1-ms pulses of transverse current at various frequencies. Photographical superposition of ink-writer records. *b*, Relationship between maximum isometric tension and stimulus frequency. Data from seven fibres, mean + range indicated. *c*, Force-velocity data from same seven fibres, each denoted by a symbol. Hyperbola drawn with constants given in text. Upper and lower hatched regions are areas encompassing all values for twitch and slow fibres, respectively⁷.

Eight transparent fibres with some light-scattering particles were studied. Seven of these gave all-or-none twitches (Fig. 1*a*) with amplitudes of 19–26% of maximum tetanic tension (mean twitch/tetanus ratio = 0.21). (The eighth fibre had a twitch/tetanus ratio of 0.02, only. It responded to high K and acetylcholine (ACh) solutions in the same way as the other fibres.) The contraction times (t_c) were 95–150 ms (mean = 117 ms), and the half-relaxation times ($t_{1/2r}$) were 110–230 ms (mean = 151 ms). The corresponding values for six type 2 twitch fibres were twitch/tetanus ratio: 0.61 (0.48–0.69); t_c : 34.3 ms (29–39); $t_{1/2r}$: 34.8 ms (30–42) (J. L., unpublished). As expected from the slow time-course of twitches, fusion during repetitive stimulation occurred at a low frequency, starting at about 5 Hz; the maximum tension was reached at about 60 Hz (Fig. 1*a, b*).

Force-velocity data obtained from isotonic shortening experiments (quick release performed at 1 s from start of tension rise) are shown in Fig. 1*c*. Although there is some scatter of the data it is readily seen that the P - V values of the present fibres are between those of twitch fibres (type 2) and slow fibres, and that the three groups of data are clearly separate. The values for the constants in Hill's equation⁹ were obtained from linear regression analysis of the data plotted in linear form; a was found to be $0.22 \pm 0.04 P_0$, b was 0.49 ± 0.12 lengths per s, and the extrapolated V_{max} was 2.17 ± 0.25 lengths per s. The mean value for V_{max} for twitch fibres was previously found to be 5.20 lengths per s and for slow fibres 1.10 lengths per s (ref. 7).

One way to distinguish between fast (twitch) fibres and slow fibres is to quickly depolarise the fibre membrane, for example by applying a solution with high K-concentration, and record the ensuing tension development. If large depolarisations are used twitch fibres produce tension for some seconds and then relax spontaneously, whereas slow fibres maintain tension much longer, often for several minutes. Figure 2*a* shows that the fibres

studied are also capable of producing long-lasting K contractures. A comparison of data obtained in 40–45 mM K show that they are in fact as 'tonic' as slow fibres; the mean tension level at 15 s from the start of depolarisation was 91% (90–92%, $n = 5$) of peak tension; corresponding values for slow fibres were 93% (83–99%, $n = 8$). At higher K concentrations (80 mM) the rate of tension decline was faster; this was also observed in slow fibres. The relationship between K concentration and peak contracture tension was similar to that of slow fibres and clearly different from that of ordinary twitch fibres (Fig. 2*b*).

The fibres studied respond with maintained contractures not only to K depolarisation but also to low doses of ACh (Fig. 2*c*). The threshold concentrations were 5×10^{-8} – 10^{-7} g per ml ACh, slightly higher than for slow fibres (Fig. 2*d*). The peak tension with ACh stimulation was the same as with K depolarisation.

Fibres stained for cholinesterase activity after completion of mechanical recordings revealed 5–6 discrete endplate regions, each 300–400 μ m in length, distributed approximately evenly along the length of the fibres (7.4–10.2 mm). In each stained region there was a loose, irregular network of branches, orientated mainly longitudinally. Branches were usually beaded with incomplete separation of beads. Figure 3*a* shows one representative endplate region. For comparison, three slow fibres were stained using the same method. These had 7–10 endplate regions varying considerably in extent. Each region consisted of an aggregate of small, stained areas, largely separate from each other (Fig. 3*b*).

Electron micrographs of two fibres showed features essentially agreeing with those described previously⁶ for 'type 4' fibres, that is wide (~ 80 nm), slightly wavy Z-bands with a square lattice as seen in cross-section, a sparse sarcoplasmic reticulum, relatively few, small mitochondria and large myofibrils with irregular outlines. The myofibril cross-sectional area was determined from electron micrographs as described previously⁶. The value for the two fibres was $1.7 \pm 1.2 \mu\text{m}^2$ ($n = 90$), and for the slow fibre it was $2.6 \pm 1.0 \mu\text{m}^2$ ($n = 50$). Smith and Ovalle found mean areas of 2.8 ± 1.2 and $5.4 \pm$

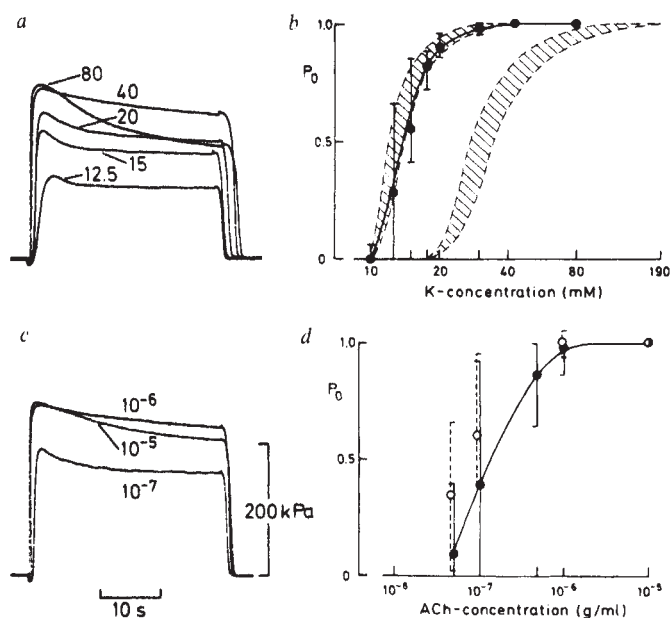


Fig. 2 Contractures elicited by high K solution (*a* and *b*) or ACh (*c* and *d*). Photographically superimposed records of responses to 30-s exposures to various test solutions in *a* and *c*. *a* and *c* are from the same fibre (as in Fig. 1*a*), taken at the same gain. Data in *b* are from six fibres, mean + range given. Right and left hand hatched areas indicate range of data for twitch and slow fibres, respectively (from ref. 10). *d*, Filled circles and bars are mean values + range for six fibres; open circles and interrupted lines give corresponding values for slow fibres (from ref. 11).

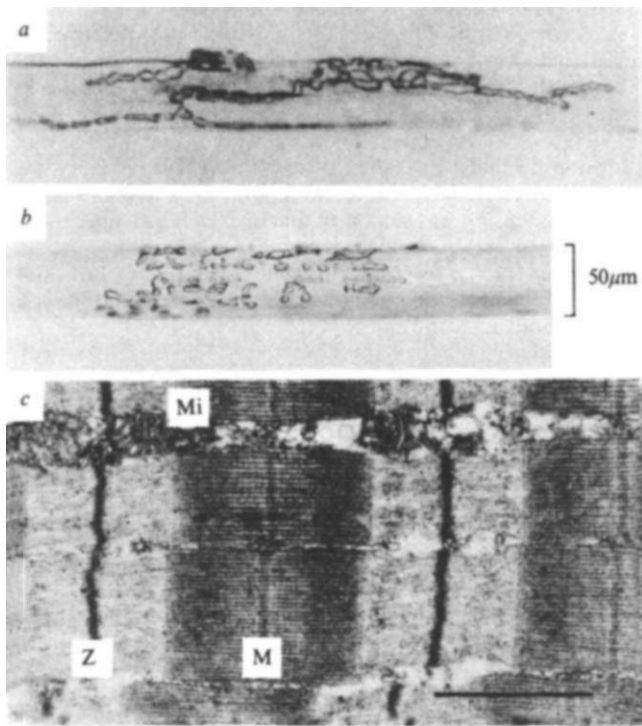


Fig. 3 *a*, One of five endplate regions on intermediate fibre, cholinesterase stain. *b*, Similar staining of a slow fibre, one of seven endplate regions shown, same magnification as in *a*. *c*, Electron micrograph, longitudinal section of an intermediate fibre. Slightly irregular Z-lines (Z), distinct M-lines (M), and small mitochondria (Mi). Scale bar, 1 μm .

2.5 μm^2 for type 4 and type 5 (slow) fibres, respectively. Thus, although the values obtained here are lower than those previously reported, it seems that the ratios of the sizes of the myofibrils are similar in the two cases. The fibres studied here may thus be classified as type 4 fibres. However, it is suggested that a more descriptive designation would be 'intermediate' fibres, which also takes into account their intermediary status with respect to functional properties.

Whether intermediate fibres form a circumscribed fibre class or whether there is in fact a continuous gradation of contraction speed and other properties cannot be determined on the basis of the present small sample. The moderate scatter of the *P-V* data makes the former suggestion appear more likely, but it must be remembered that fibres which had a well-defined microscopic appearance were selected for this study.

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J. LÄNNERGREN

Department of Physiology II,
Karolinska institutet,
S-104 01 Stockholm 60, Sweden

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Requirements for *in vitro* shortening and lengthening of isolated thick filaments of *Limulus* striated muscle

DE VILLAFRANCA¹ first reported that the A bands of *Limulus* skeletal muscle shortened during ATP-stimulated contraction of glycerinated myofibrils. We have found that in *Limulus* striated muscle, unlike vertebrate striated muscle, the length of the A band varies linearly with sarcomere length². This is partly due to variation in thick filament length. We have isolated either long or short thick filaments from this muscle, depending on the conditions to which the muscle was exposed before the isolation procedure³. We have further determined that the paramyosin-containing thick filaments^{4,5} of the *Limulus* telson muscle decrease 30-40% in length² and increase in diameter by a similar amount during sarcomere shortening below rest length (7.0 μm)⁶. The mechanism for the control of this shortening process is unknown but the series of experiments reported here implicate Ca^{2+} and ATP in thick filament shortening.

Isolated thick filaments were prepared from the medial telson levator muscle, stretched to its *in vivo* rest length. The muscle was soaked in 50% glycerol-50% relaxing solution at 4°C overnight (5 mM Tris buffer, 100 mM KCl, 1 mM dithiothreitol, 5 mM MgCl_2 , 8 mM EGTA, 5 mM ATP, pH 7.0). In this and all subsequent solutions tetracycline was added to minimise bacterial contamination. The next day several fibres were dissected free and the sarcomere length determined for the bundle by laser diffraction.

Following treatment in relaxing solution, the bundle was homogenised in 10 volumes of relaxing solution containing 0.4 mg ml^{-1} electrophoretically pure DNase 1 (Sigma), 1% phenylmethyl sulphonyl fluoride (PMSF) and 0.1 M dimethyl sulfoxide (DMSO) and stirred for 4 h at 4°C. DNase 1 has been shown to bind in a mol: mol ratio with g-actin⁷ giving a relatively actin-free preparation of thick filaments following differential centrifugation. PMSF/DMSO, an effective protease inhibitor, was used to reduce the effects of any protease impurities in the DNase. The homogenate was then mixed with an equal volume of DEAE cellulose in relaxing solution. Most remaining f-actin selectively absorbed to the DEAE cellulose⁸.

Three low speed (3,000 r.p.m.) centrifugations, recovering the supernatant, followed by three high speed (30,000 r.p.m.) centrifugations yielded a pellet of thick filaments, which were resuspended in 10 volumes of relaxing solution. Isolated filaments thus obtained were negatively stained with uranyl acetate on Formvar coated grids and their length determined from electron photomicrographs. Only filaments with a middle bare zone and tapered ends were measured. These filaments suspended in a low Ca^{2+} ($<1 \times 10^{-8}$ M) solution averaged $4.3 \mu\text{m} \pm 0.3$ in length (Table 1). Differences between filament lengths of control and treated samples were analysed by the Student's 2-sample, 2-tailed *t* test.

To examine the effects of Ca^{2+} , the filaments were centrifuged and resuspended in relaxing solution containing a Ca^{2+} /EGTA ratio of 1 (8 mM Ca^{2+} , 8 mM EGTA, estimated free Ca^{2+} 8×10^{-6} M). After several minutes and up to 4 h, these filaments were negatively stained and their length measured. The isolated filaments were significantly shorter ($3.4 \mu\text{m} \pm 0.6$, $P < 0.001$) (Table 1). Filaments were maximally shortened after as little as 1 min in the Ca^{2+} -ATP solution. Thus it appears that Ca^{2+} at physiological levels⁹ triggers thick filament shortening.

In a second series of experiments designed to examine the specific requirement for ATP in thick filament shortening, ATP was replaced with a nonhydrolysable analogue, adenylyl imidodiphosphate (AMP-PNP)¹⁰. Thick filaments isolated by the procedure described above, in low Ca^{2+} -AMP-PNP solution, were long ($4.0 \mu\text{m} \pm 0.3$). The filaments were centrifuged and resuspended in a solution containing a Ca^{2+} /EGTA ratio of

1 and 5 mM AMP-PNP, maintained for 4 h and measured again. There was no significant change in the length of filaments in the solution containing Ca^{2+} and AMP-PNP ($4.0\mu\text{m} \pm 0.3$) (Table 1).

These experiments strongly suggest that Ca^{2+} , which mediates the actin-myosin interaction in these muscles¹¹⁻¹⁴ also regulates thick filament shortening. ATP is also required, as shortening of the thick filaments does not occur in very low ATP or when the analogue is present. The levels of Ca^{2+} and ATP required for *in vitro* shortening parallel those that are thought to mediate excitation-contraction coupling^{9,15}. While in these experiments some actin was present, it was significantly less than *in vivo*. This suggests that actin may not be necessary for regulation of the thick filament length.

A Ca^{2+} -dependent phosphorylation of contractile proteins has been observed in both smooth and striated muscles of a number of species¹⁶⁻²³. Because of these findings we decided to investigate the effect of alkaline phosphatase on the isolated thick filament preparation. Thick filaments were isolated long ($4.1\mu\text{m} \pm 0.3$) and then shortened in $\text{Ca}^{2+}/\text{EGTA} = 1$, 5 mM ATP solution ($3.0\mu\text{m} \pm 0.5$). One aliquot of these shortened thick filaments was resuspended in relaxing solution containing 0.4 mg ml^{-1} alkaline phosphatase (Worthington) (1% PMSF/0.1 M DMSO) for 3 h. Alkaline phosphatase, isolated from chicken intestine, is a relatively nonspecific phosphomonoesterase which catalyses hydrolysis of acyl phosphates²⁴. A second aliquot was resuspended in low Ca^{2+} relaxing solution without alkaline phosphatase. The filaments examined following treatment with alkaline phosphatase had lengthened to $3.7\mu\text{m} \pm 0.4$, $P < 0.001$ (Table 1). Shortened filaments returned to the standard low Ca^{2+} relaxing solution in the absence of alkaline phosphatase remained short, $3.1\mu\text{m} \pm 0.4$. The Ca^{2+} -ATP induced shortening appeared to be reversible by the addition of alkaline phosphatase (Fig. 1).

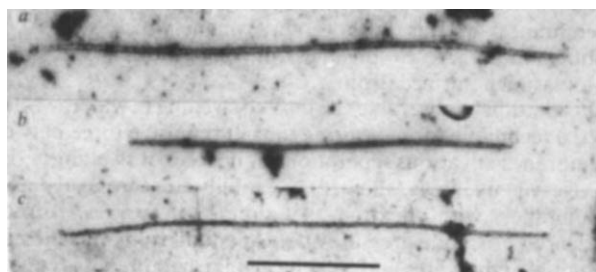


Fig. 1 Thick filaments. *a*, Isolated from $7.0\mu\text{m}$ sarcomere in standard relaxing solution; *b*, following treatment with a solution containing a $\text{Ca}^{2+}/\text{EGTA} = 1$ (8 mM Ca^{2+} , 8 mM EGTA estimated free Ca^{2+} , $8 \times 10^{-6}\text{ M}$) and 5 mM ATP; *c*, after incubation for 3 h with 0.4 mg ml^{-1} alkaline phosphatase in standard relaxing solution $\times 44,000$; scale bar, $1\mu\text{m}$.

In the last series of experiments, filaments isolated long ($3.8\mu\text{m} \pm 0.3$) were shortened in the presence of Ca^{2+} and ATP ($3.1\mu\text{m} \pm 0.3$, $P < 0.001$) treated with alkaline phosphatase ($3.5\mu\text{m} \pm 0.4$, $P < 0.001$), reshortened with Ca^{2+} and ATP ($3.2\mu\text{m} \pm 0.3$, $P < 0.001$) and finally retreated with alkaline phosphatase ($3.5\mu\text{m} \pm 0.3$, $P < 0.001$) (Table 1).

As isolated thick filaments from *Limulus* could be lengthened repeatedly following treatment with alkaline phosphatase, we feel our results are consistent with a dephosphorylation mechanism for the lengthening of shortened thick filaments. Experiments are presently underway to examine whether phosphorylation may be the mechanism for thick filament shortening.

Finally, Davidheiser *et al.*²⁵ have compared the work efficiency of *Limulus* telson muscle allowed to contract at two initial muscle lengths; long (sarcomere $\sim 9.5\mu\text{m}$) and short

Table 1 Length of thick filaments

Experimental procedure*	Thick filament length (μm) ($\bar{x} \pm 1\text{ s.d.}$)
1. No Ca^{2+} , EGTA, ATP $\text{Ca}^{2+}/\text{EGTA} = 1$, ATP	4.3 ± 0.3 ($n = 150$) 3.4 ± 0.6 ($n = 123$)†
2. No Ca^{2+} , EGTA, AMP-PNP $\text{Ca}^{2+}/\text{EGTA} = 1$, AMP-PNP	4.0 ± 0.3 ($n = 66$) 4.0 ± 0.3 ($n = 53$)
3. No Ca^{2+} , EGTA, ATP $\text{Ca}^{2+}/\text{EGTA} = 1$, ATP Alkaline phosphatase, ATP, EGTA, No Ca^{2+}	4.1 ± 0.3 ($n = 200$) 3.0 ± 0.5 ($n = 141$)† 3.7 ± 0.4 ($n = 88$)†
4. No Ca^{2+} , EGTA, ATP $\text{Ca}^{2+}/\text{EGTA} = 1$, ATP Alkaline phosphatase, ATP, EGTA, No Ca^{2+} $\text{Ca}^{2+}/\text{EGTA} = 1$, ATP Alkaline phosphatase, ATP, EGTA, No Ca^{2+}	3.8 ± 0.3 ($n = 115$) 3.1 ± 0.3 ($n = 146$)† 3.5 ± 0.4 ($n = 53$)† 3.2 ± 0.3 ($n = 66$)† 3.5 ± 0.3 ($n = 52$)†

The mean values of thick filaments isolated long vary because experiments 1 to 4 were performed on different preparations of thick filaments.

* Concentrations were: Ca^{2+} , 8 mM; EGTA, 8 mM; ATP, 5 mM; AMP-PNP, 5 mM; Alkaline phosphatase, 0.4 mg ml^{-1} , MgCl_2 , 5 mM; KCl, 100 mM.

† Statistically significant difference between value marked and preceding value ($P < 0.001$).

(sarcomere $\sim 4.5\mu\text{m}$). They found a 50% decrease in efficiency (defined as work/phosphoarginine utilisation) when the starting length of the muscle was short. It is possible that the decreased efficiency resulted from phosphate previously used to activate actin-myosin interaction being diverted to thick filament shortening.

L. BRANN
M. M. DEWEY
E. A. BALDWIN
P. BRINK
B. WALCOTT

Department of Anatomical Sciences,
Health Sciences Center,
State University of New York at Stony Brook,
Stony Brook, New York 11794

Received 30 August 1978; accepted 16 March 1979.

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Myelin swelling and measurement of forces between myelin membranes

MYELIN has been observed by many workers to swell to a limited extent in water^{1,2}; a swelling thought to result from electrostatic repulsion between the myelin membranes¹. The extracellular space between membrane pairs increases by approximately 80 Å on immersion in water, whereas the intracellular space changes by only a few angstroms^{1,2}. On the other hand, in phospholipid bilayer systems, very low surface charge densities (approximately 2 mol per cent charged phospholipid or 1 charge per 3,500 Å²) cause otherwise neutral phospholipid bilayers, which normally swell to a very limited extent, to separate indefinitely in water as a result of electrostatic repulsion^{3,4}. At even lower surface charge densities, intermediate bilayer separations between that of neutral bilayers (about 27 Å) and charged bilayers (indefinite swelling) have rarely, if ever, been observed. Why, then, does nerve myelin swell to only a limited extent in water? We describe here our attempts to answer this question, and report that myelin is prevented from swelling indefinitely by mechanical forces. We have also succeeded in measuring the net repulsive force between myelin membrane pairs.

As the myelin sheaths and even individual Schwann cell sheaths are surrounded by a connective tissue matrix, we considered it possible that the limited swelling resulted from the myelin membranes being mechanically restrained. In fact, Worthington and Blaurock¹ have suggested that connective tissue might tend to prevent myelin from swelling. Thus, when myelin is placed in pure water the membrane separation will increase under the influence of an electrostatic repulsive pressure, P_{ES} , until the membranes are pressed against or restrained by connective tissue such as the collagenous basement membrane (Fig. 1). At the myelin membrane separation where further swelling is prevented by this connective tissue, a hydrostatic pressure, P_{HS} , will build up within the myelin. At equilibrium, the chemical potential of the water between the myelin membranes, μ_w^m , must be equal to that of pure water. This is because the negative pressure generated by the electrostatic repulsion between the membranes is equal to the positive hydrostatic pressure developed as a result of the connective tissue constraint; the net pressure ($P_{ES} - P_{HS}$) is zero. Digestion of the connective tissue should therefore allow the membranes to separate indefinitely.

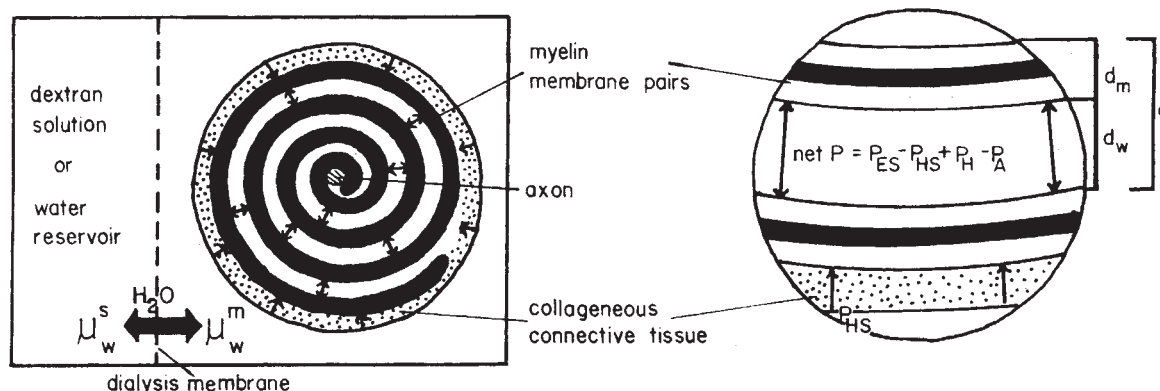


Fig. 1 Schematic representation of the forces acting at the myelin membrane.

$$\mu_w^m = -(P_{ES} + P_H - P_{HS} - P_A) \bar{V} \text{ (erg mol}^{-1}\text{)}$$

For $d_w > 30$ Å, P_H and P_A are negligible (4), μ_w^m becomes $-(P_{ES} - P_{HS}) \bar{V}$: $\mu_w^s = -(P_\pi) \bar{V}$ (erg mol⁻¹) ($P_\pi = 0$ for water). At equilibrium $\mu_w^s = \mu_w^m$

$$P_\pi = P_{ES} - P_{HS} \text{ (dyn cm}^{-2}\text{)}$$

(in water $P_{ES} = P_{HS}$)

where: μ_w^m = chemical potential of water between myelin membranes; μ_w^s = chemical potential of water in solution; $\bar{V}$ = molar volume of water (cm³ mol⁻¹); P_{ES} = electrostatic repulsive pressure; P_{HS} = hydrostatic pressure within the myelin; P_H = hydration repulsive pressure (4); P_A = van der Waals attractive pressure (4); P_π = osmotic pressure of dextran solution.

Table 1 Lamellar repeat spacings for frog sciatic nerve, measured by X-ray diffraction

	Tied lengths	Untied fragments	Collagenase treated
Ringer's solution	174.1–175.7 (4)	175.8–180.1 (5)	173.8 (1)
Water or buffer	230.6–252.0 (4)	Central scatter (4)	Central scatter (1)

The numbers give the full range of lamellar repeat spacings in Å, obtained by X-ray diffraction, for frog sciatic nerve treated three different ways and suspended in either normal amphibian Ringer's solution or in water or dilute buffer. The number of samples is given in parentheses. Central scatter indicates loss of lamellar order and suggests indefinite swelling of the myelin structure.

We succeeded in disrupting the connective tissue in myelin without detectably perturbing the membrane structure, and in so doing observed indefinite swelling and separation between myelin membranes in water. We then measured the net repulsive force between these unrestrained myelin membranes as the distance between them was varied by manipulating the chemical potential of the water in the bathing medium^{6,12}.

We used X-ray diffraction to observe tied lengths, untied fragments (1–2 mm long) and collagenase-treated samples of frog sciatic nerve. The collagenase treatment consisted of a 2-h incubation at room temperature in Ringer's solution containing 0.1% collagenase, and was designed to break down collagen fibres (1–4-h incubation gave identical results). Initially, all three kinds of samples were observed first in normal Ringer's solution and then in water or buffer (0.001 M Tris or phosphate buffer, pH 6.8). The X-ray results indicate that myelin is a lamellar structure with a total repeat distance, d , which has been shown to include an asymmetrical pair of membranes separated by cytoplasmic and extracellular aqueous layers². To estimate the separation, d_w , between the extracellular faces of the myelin membranes (as this is the compartment which swells¹), we subtracted 155 Å, the thickness of a membrane pair plus the cytoplasmic interface, from d .

In addition to observing the limited swelling in water, we have used a technique for measuring the net repulsive force between membranes at various separations or degrees of swelling^{5,6} (Fig. 1). Nerve myelin is allowed to equilibrate across a dialysis membrane, with dextran solutions of various, directly measured⁶, osmotic pressures, P_π . At equilibrium, the chemical potential of the water between the myelin membranes is equal to

that of the dextran solution. Thus, the net repulsive pressure between myelin membranes is equal to the external osmotic pressure, P_w . Electrostatic repulsion P_{ES} , van der Waals attraction P_A , hydration forces P_H , and, if the myelin is restrained mechanically by connective tissue, hydrostatic pressure P_{HS} , all contribute to the net repulsive pressure between membranes, and affect swelling. At membrane separations greater than 30 Å, P_H and P_A are negligible compared with P_{ES} and P_{HS} (ref. 4).

Table 1 shows that in Ringer's solution, all three kinds of sample have similar spacings that are equivalent to published measurements^{1,2} (~175 Å). Visual inspection shows that the X-ray intensities in Ringer's solution are also identical to those in the literature. Thus, cutting the nerves into fragments or treating them with collagenase changes neither the myelin membrane separation nor its structure. When placed in water or buffer, intact tied nerve swells to a limiting value of ~240 Å, entirely consistent with the work of others^{1,2}. On the other hand, untied fragments and collagenase-treated nerves seem to swell indefinitely, becoming disordered in water. This disordering, shown by central scattering of the X rays, is characteristic of model membranes that swell indefinitely⁴ and is reversible on re-immersion of the myelin in Ringer's solution. The presence of central scattering by the treated nerves only suggests indefinite swelling; we investigated the swelling characteristics further by osmotic pressure experiments.

Figure 2 shows the results of swelling myelin against dextran solutions. At the higher osmotic pressures exerted by 35 and 40% dextran, which dehydrate myelin, two phases are observed; a normal Ringer's phase and a faint phase which may be interpreted as corresponding to the compact phase observed by Kirschner *et al.*⁹⁻¹¹ in other compacting conditions.

As the osmotic pressure of the dextran solution decreases, tied myelin swells to its limiting value of approximately 240 Å. Further reduction of the osmotic pressure produces no further change in spacing. The maximum osmotic pressure which gives this limiting value is equal to the hydrostatic pressure sustained

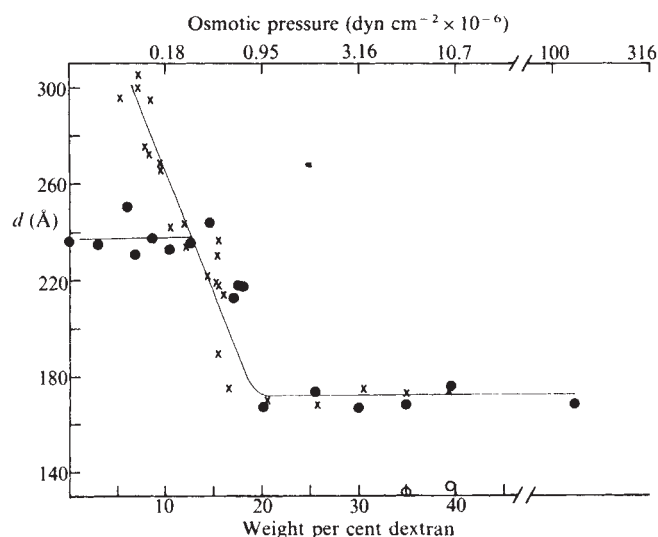


Fig. 2 A plot of the repeat spacing of myelin equilibrated against different concentrations (therefore different osmotic pressures) of dextran in 0.001 M phosphate or Tris buffer, pH 6.8. As the weight per cent dextran and the osmotic pressure decreases, tied lengths of myelin (●) swell to a limited extent (to about 240 Å) while collagenase-treated myelin (×) swells to 300 Å before disorder precludes accurate measurements of its spacing. The maximum swelling of the restrained intact myelin, at approximately 15 wt% dextran, indicates that in water it must offset this osmotic pressure of 4×10^5 dynes cm^{-2} with an equal internal hydrostatic pressure. The second lamellar phase (○) observed in 'dehydrated myelin' (35 and 40% dextran) indicates a phase separation first described by Kirschner *et al.*⁹. (The highest pressure point was obtained using vapour pressure rather than osmotic pressure¹².)

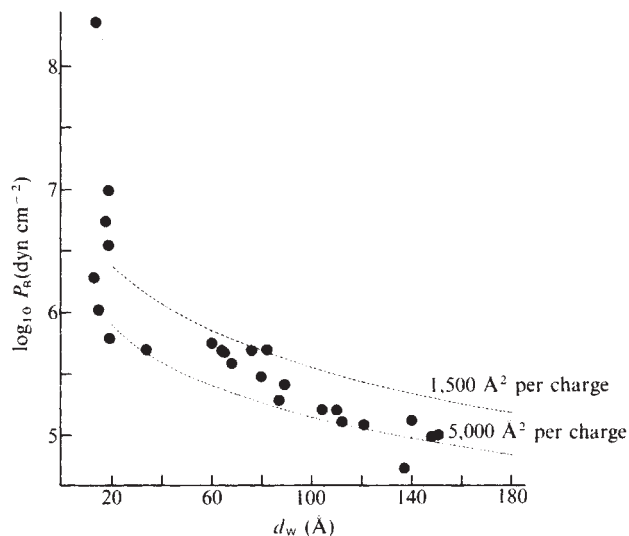


Fig. 3 A plot of the net repulsive pressure between extracellular faces of myelin membranes as a function of their separations. (The highest pressure point (at $\log P = 8.35$) was obtained using vapour pressure rather than osmotic pressure¹².) The enormous repulsion at close separation is indicative of hydration forces⁶ whereas the weaker repulsion at 40–140 Å separation is indicative of electrostatic repulsion⁴. The dashed lines show the theoretical electrostatic repulsion expected on the basis of the double-layer theory for charge densities of 1,500 Å² per charge and 5,000 Å² per charge.

by the tied myelin in water. Figure 2 shows this pressure to be, at a maximum, about 4×10^5 dyn cm^{-2} or ~0.4 atm. Biological tissues can easily withstand this stress. For example, such a pressure would generate a tension of 20×10^3 dyn cm^{-1} in a 1-mm diameter connective tissue collar around the myelin (less for smaller diameters). This maximum tension is an order of magnitude less than tensions generated in other biological tissues such as blood vessel walls (17×10^4 dyn cm^{-1} (ref. 7) or frog sarcoplasmic reticulum (20×10^4 dyn cm^{-1} (ref. 8)).

Variable dimensions obtained using untied fragments in the osmotic pressure experiments suggested that this method did not consistently remove all constraint associated with the intact nerve structure and we have therefore reported only those experiments done on collagenase-treated nerves (Fig. 2). Against the higher osmotic pressures exerted by 20–40% dextran solutions, treated myelin samples seem to be identical to intact myelin. However, as the osmotic pressure is decreased further, the collagenase-treated samples continue to swell systematically well beyond the limiting value for tied nerve, reaching approximately 300 Å against an osmotic pressure of 10^5 dyn cm^{-2} . Beyond this point, the X-ray reflections become broad and diffuse, indicating increasing disorder in the myelin array.

These data may now be used to construct the relationship between repulsive pressure between membranes and membrane-pair separation, d_w . To establish this relationship to a first approximation, we have assumed, as has been observed by others¹, that the thickness of the membrane pair plus cytoplasm is relatively constant and equal to 155 Å, and that the change in spacing on swelling is due to a change in the extracellular compartment. (Although this is not strictly the case, as shown by Worthington and Blaurock¹, the change in the cytoplasmic spacing (approximately –6 Å) is small compared with that in the extracellular compartment (80–130 Å).) The force relationship shown in Fig. 3 is then obtained by plotting the net repulsive pressure between membrane pairs, P_w , against membrane-pair separation ($d_w = d - 155$ Å). This relationship is very similar to that obtained for certain charged phospholipid bilayers⁴. Theoretical curves derived from the electrostatic double-layer theory⁴ and for surface charge densities of 1,500 Å² and 5,000 Å² per charge are shown in Fig. 3 by dotted lines. The

weaker repulsion seen at large separations is consistent with the electrostatic repulsion theory⁴. The enormous repulsion pressures observed at lower separations are similar to the hydration pressures observed between phospholipid membranes⁵, but in the present case might be accounted for by 'steric' hindrance between membranes.

We therefore conclude that a mechanical restraint, first pointed out by Worthington and Blaurock¹, prevents nerve myelin from swelling indefinitely in water and can be removed by collagenase without perturbing the membrane structure. By removing this restraint and applying a technique of osmotically stressing the myelin structure, we have measured the net repulsive force between myelin membrane pairs. This will make possible a more detailed investigation of the nature of the interaction between myelin membranes of various kinds.

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R. P. RAND
N. L. FULLER
L. J. LIS

Department of Biological Sciences,
Brock University, St. Catharines,
Ontario L2S 3A1, Canada

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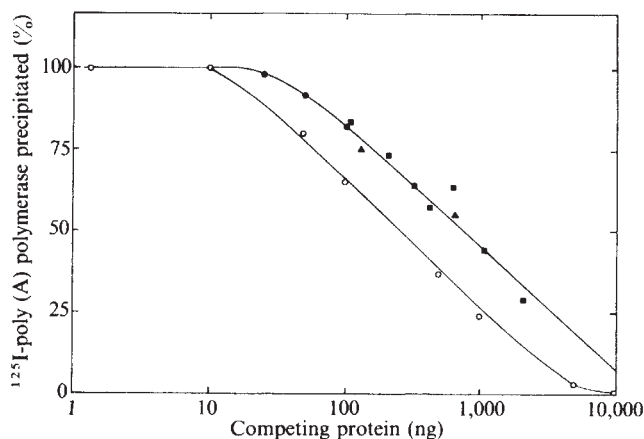


Fig. 1 Competition radioimmunoassay. Poly(A) polymerase was purified from nuclei of Morris hepatoma 3924A as described previously¹⁹ and subjected to affinity chromatography on DNA-cellulose³⁴. Purified enzyme was iodinated with Na¹²⁵I and Chloramine T essentially as described by Greenwood *et al.*³⁵. An IgG fraction was obtained from serum of rabbits injected four times at biweekly intervals with purified enzyme emulsified in Freund's adjuvant. A crude IgG fraction was prepared by (NH₄)₂SO₄ precipitation and a 1:10,000 dilution was used, which complexed with 10-20% of the input antigen (20,000 c.p.m.). ¹²⁵I-poly(A) polymerase: IgG complexes formed over a 72-h period at 4 °C, were incubated (4 h at 4 °C) with immobilised second antibody (goat-antirabbit IgG) and recovered by centrifugation. The competition radioimmunoassay was carried out by incubating unlabelled antigen with antibody for 4 h at 4 °C prior to addition of ¹²⁵I-poly(A) polymerase and further incubation for 72 h. The competition radioimmunoassay was specific for poly(A) polymerase; addition of unrelated proteins caused no reduction in the amount of ¹²⁵I-enzyme recovered in the complex. Results are expressed as per cent of control with the 100% value (equal to 2,000 c.p.m.) representing the amount of ¹²⁵I-poly(A) polymerase recovered in the absence of competing antigen. HeLa cell polysomal fraction was prepared by centrifugation through sucrose gradients containing 0.5 M NaCl, essentially as described by Kumar and Pederson⁵, and resuspended in RNase buffer containing 0.5 M NaCl, 0.01 M Tris-HCl, pH 7.4. The ribonuclease resistant fractions, following digestion with 10 µg ml⁻¹ pancreatic and 40 units per ml T₁ ribonucleases for 75 min at 37 °C, were fractionated on an oligo(dT) cellulose column. The poly(A)-ribonucleoprotein fractions bound to the oligo(dT) column were eluted with 0.5 M NaCl, 0.01 M sodium phosphate buffer, pH 7.0, containing 25% formamide. After concentration by centrifugation, proteins were resolved by polyacrylamide gel electrophoresis as described by Laemmli³⁶. The protein of MW 75,000, identified by parallel molecular weight marker proteins, was excised and eluted in 0.01 M phosphate buffer and concentrated by lyophilisation. Protein was determined by the methods of Bennett³⁷ and Schaffner and Weissman³⁸ using bovine serum albumin as standard. The average of the results of the two protein estimations was used to compute the input protein. Experimental values represent the mean of quadruplicate samples. The slopes of the lines were computed by regression analysis using values in the linear range of the semi-log plot. O, Purified poly(A) polymerase; ●, ■, ▲, purified P75, values from three separate experiments.

Poly(A) polymerase and poly(A)-specific mRNA binding protein are antigenically related

THE polysomal mRNA of eukaryotes is associated with at least two tightly binding proteins of approximate molecular weights 75,000 and 60,000¹⁻⁵. The 75,000 MW polypeptide (P75) is specifically bound to the poly(A) portion of the mRNA^{2,6,7}. Heterogeneous nuclear RNA molecules (HnRNAs), which apparently contain the precursors to the cytoplasmic mRNAs, are also associated with several polypeptides^{5,8}. Although the array of proteins bound to HnRNAs is more complex than that bound to mRNAs, one major protein of MW 75,000 is associated with the poly(A) tracts in the HnRNA⁸. It has been postulated that the nuclear P75 is identical to the cytoplasmic polypeptide of the same size⁸. It has also been suggested that P75 is responsible for the transport of poly(A)-containing mRNAs from nucleus to cytoplasm⁹. However, direct evidence for the function of P75 has been lacking. While characterising

poly(A) polymerase (EC 2.7.7.19), the enzyme responsible for the post-transcriptional addition of poly(A) to the 3' terminus of the mRNA, we observed several similarities with P75. In particular, both occur in the nucleus in soluble and bound states¹⁰⁻¹², can be isolated from polysomes^{2,5,13,14} or post-microsomal cytoplasm¹³⁻¹⁸, have almost identical amino acid compositions^{13,19}, and have similar MWs of 75,000 and 60,000 (refs 19, 20), respectively. Unfortunately, the conditions required for P75 purification have precluded determination of any associated enzyme activity. We have circumvented this difficulty by raising antibody to poly(A) polymerase, and developing a sensitive and specific radioimmunoassay for this enzyme. We now report that

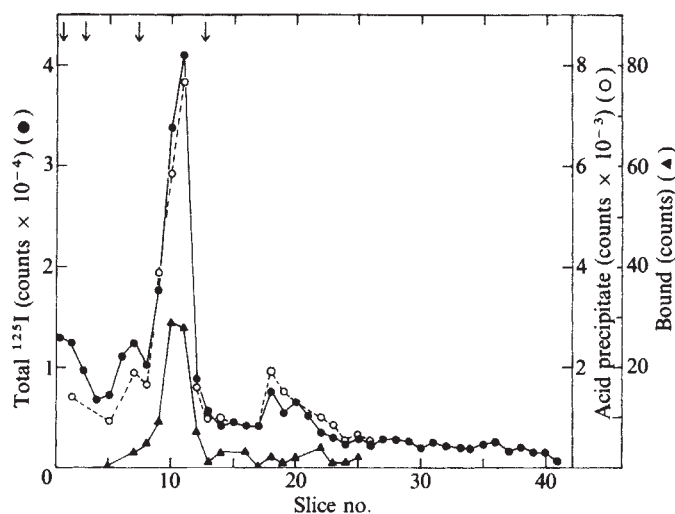


Fig. 2 Polyacrylamide gel electrophoresis of ^{125}I -P75. P75 was purified essentially as described in Fig. 1 and iodinated using Chloramine T as adapted for poly(A) polymerase. ^{125}I -P75 (250,000 c.p.m.) was denatured in buffer containing 100 mM sodium phosphate pH 7.1, 0.7% (w/v) SDS and 10 mM β -mercaptoethanol. Denatured protein was then subjected to polyacrylamide gel electrophoresis as described previously¹⁹ except that the separating gel (9 cm) was 10% acrylamide (with 2.7% cross-linking) and was overlaid with a stacking gel (1 cm) of 3% acrylamide (2.7% cross-linked). Standard marker proteins were run in parallel. After electrophoresis, the gel containing the iodinated sample was frozen, then sliced and total radioactivity of each slice determined in a gamma counter. Protein was then eluted from each slice by mechanical shearing followed by addition of 500 μl 10 mM sodium phosphate pH 7.5, and incubation for 18 h at 4 °C. One half of the eluate from each slice was precipitated by addition of 2 volumes of 10% trichloroacetic acid followed by centrifugation. After washing the pellet twice with 5% acid, the radioactivity was determined. The other half of the eluted sample was incubated for 3 d at 4 °C with the immunoglobulin fraction obtained from hepatoma poly(A) polymerase-injected rabbits. The ^{125}I -P75 bound to antibody was recovered by incubation with second antibody as described in Fig. 1. ●, Total radioactivity; ○, acid-precipitable radioactivity; ▲, radioactivity recovered in the immune complex. Results are expressed as counts in 2 min. Arrows indicate position of migration of the standard proteins (from left to right), myosin, β -galactosidase, phosphorylase B and bovine serum albumin.

P75 can form complexes with antibodies to poly(A) polymerase and can compete with this protein in the radioimmunoassay. These data suggest that the two polypeptides are structurally similar and, possibly, identical.

Poly(A) polymerase was purified essentially to homogeneity from nuclei of a rapidly growing rat tumour, Morris hepatoma 3924A, as described by Rose and Jacob¹⁹. Antibodies were produced in rabbit and a competition radioimmunoassay was developed to detect nanogramme quantities of enzyme. The radioimmunoassay was specific for poly(A) polymerase based on the following criteria: (1) several other proteins including nucleic acid polymerising enzymes did not compete with poly(A) polymerase in the assay and (2), the amount of poly(A) polymerase (μg) per g tissue measured by radioimmunoassay in partially purified enzyme preparations agreed well with our published data¹⁹ on the amount (μg per g) of enzyme recovered in homogeneous preparations. (Details of the immunology of poly(A) polymerase will be presented elsewhere.) Figure 1 shows the competition radioimmunoassay between purified poly(A) polymerase and ^{125}I -poly(A) polymerase for antigen sites on the antibodies. Increasing levels of purified hepatoma poly(A) polymerase could effectively compete with radiolabelled enzyme, thereby reducing the amount of radioactivity recovered in the immune complex. With 250 ng of unlabelled poly(A) polymerase (4 pmol of enzyme, MW 60,000) a 50% reduction in recovery of ^{125}I -poly(A) polymerase was observed. P75 isolated and purified from HeLa cell polysomal mRNA⁵ could also compete for antigen sites on the antibody. A 50% reduction in ^{125}I -poly(A) polymerase bound to antibody was observed at approximately 800 ng of P75 (10 pmol of P75, MW 75,000). There was no significant difference in the slopes of the competition lines produced by increasing levels of poly(A) polymerase or P75, indicating that these two proteins have similar, and, perhaps, identical antigenic determinants. No detectable proteolysis of radiolabelled P75 occurred* in the conditions of the radioimmunoassay. Therefore, it is unlikely that the slight displacement of the competition curve for P75 relative to that of poly(A) polymerase was due to contamination of P75 with protease, which could lead to reduction in the number of antigenic sites on this protein. It is possible that the displacement of the P75 competition curve from poly(A) polymerase could be due to inactivation of antigenic sites on P75 during its purification or to post-translational modifications of P75 *in vivo* which could render this polypeptide less antigenically active. In any case, P75 was the only protein tested (besides

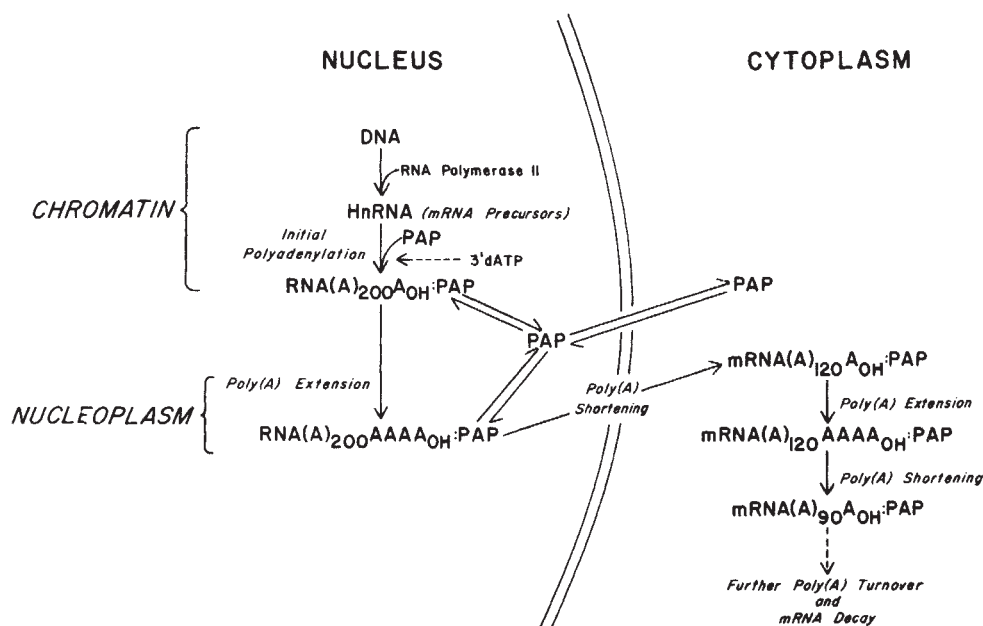


Fig. 3 Diagram depicting the association of poly(A) polymerase (PAP) with RNA throughout the processing reactions.

poly(A) polymerase itself) with the capacity to compete with iodinated poly(A) polymerase in the radioimmunoassay. The specificity of P75 was substantiated by carrying out the competition radioimmunoassay with a fraction containing all the mRNA binding proteins. In such an experiment, larger quantities of protein from the less pure preparation than of purified P75 were required to displace iodinated poly(A) polymerase from the immune complex. The decreased capacity of the total mRNA binding proteins to compete in the radioimmunoassay was consistent with the reduction in the net content of P75 in that fraction and substantiated our hypothesis that the radioimmunoassay is specific for P75/poly(A) polymerase.

The ability of P75 to interact with antibodies produced against poly(A) polymerase was further confirmed by demonstrating that P75 itself could be recovered in the immune complex. This was accomplished by iodinating P75 and subjecting it to polyacrylamide gel electrophoresis in denaturing conditions. Figure 2 shows that the bulk of the iodinated sample migrated at 75,000 MW and was precipitable with trichloroacetic acid, demonstrating both the purity of the sample and the covalent attachment of ^{125}I by iodination. The peak of radioactivity recovered in the immune complex was coincident with the ^{125}I -P75 peak. The presence of P75 in the immune complex further strengthens the tenet that this polypeptide is antigenically related to poly(A) polymerase.

The polyadenylation of mRNA precursors in eukaryotes occurs by rapid addition of 150–250 AMP residues²¹, most probably by the chromatin-bound poly(A) polymerase²². En route to the cytoplasm, a slow poly(A) chain extension occurs²³ presumably catalysed by the soluble nuclear enzyme²² or enzyme released from chromatin¹¹. The chromatin-bound and soluble nuclear poly(A) polymerase seem to be the same polypeptide in two functional states¹¹. In the cytoplasm, the poly(A) chain on the mRNA is reduced in size²⁴. Concomitantly, additional AMP residues are added to the 3'OH terminus²⁵. Thus, the length of the poly(A) on the mRNA at any particular time represents a balance between synthesis and degradation. The kinetic^{11,17,19} and antigenic (K. M. R., unpublished observations) similarities of the enzyme examined from different cell fractions suggest that a single polypeptide catalyses both the initial and subsequent polyadenylation of mRNA. This concept is strengthened by the present demonstration that the protein, P75, associated with polysomal mRNA, is antigenically similar to nuclear poly(A) polymerase. Studies with the drug, cordycepin (3' deoxyadenosine), have also indicated a relationship between nuclear and cytoplasmic events. Cordycepin (*in vivo*) and cordycepin 5' triphosphate (*in vitro*) have been shown to cause a reduction in the initial polyadenylation of nuclear precursors^{21,22} catalysed by chromatin-associated poly(A) polymerase. This drug also causes a reduction of newly synthesised P75 bound to mRNA appearing in the cytoplasm⁹.

Although P75 and poly(A) polymerase are antigenically similar, these proteins have slightly different MWs (75,000 and 60,000, respectively). This difference is not surprising in view of the wide MW range (42,000–120,000) reported for purified poly(A) polymerase preparations from various sources²⁰. In particular, poly(A) polymerase purified from HeLa cell cytoplasm²⁶ is the same size as the HeLa cell mRNA binding protein (MW 75,000). Nuclear poly(A) polymerase from HeLa has a MW of 50,000 (ref. 26), somewhat smaller than the nuclear hepatoma enzyme used in the present studies. It is possible that the smaller poly(A) polymerases are cleavage products of larger polypeptides, and that both antigenic and kinetic properties have been retained on cleavage. Such cleavage reactions are known to occur in the nuclei of various tissues and cells^{27,28}.

The similarities between the P75 protein and poly(A) polymerase—(1) antigenic properties, (2) amino acid composition, (3) subcellular distribution and (4) sensitivity to cordycepin/cordycepin 5' triphosphate—suggest that the poly(A)-binding protein associated with mRNA and poly(A) polymerase are the same polypeptide. The function of P75 protein must, therefore, reside in the processing of mRNA and its precursor by

polyadenylation. The affiliation of the binding protein, poly(A) polymerase, with RNA throughout the polyadenylation process is outlined in Fig. 3. According to this scheme, poly(A) polymerase (P75) becomes associated with the newly synthesised mRNA precursor in the nucleus and catalyses the initial polyadenylation reaction while still bound to the chromatin. The enzyme then escorts the mRNA through the nucleoplasm and finally into the cytoplasm, continuously catalysing the poly(A) extension reaction. Although initial polyadenylation may play a part in transport of mRNA to the cytoplasm, chain extension may stabilise the mRNA by protecting it from degradation^{24,29–31}. The soluble polypeptide may be in equilibrium with the bound form and may serve as a source of readily available enzyme, both in the nucleus and in the cytoplasm. Because of the close association of poly(A) polymerase with mRNA, it now seems highly likely that alterations in the levels of poly(A) polymerase observed under different physiological and pathological conditions^{19,32,33} could be responsible for modifications in mRNA processing by polyadenylation.

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KATHLEEN M. ROSE

SAMSON T. JACOB

Department of Pharmacology
and Specialized Cancer Research Center,
The Milton S. Hershey Medical Center,
The Pennsylvania State University College of Medicine,
Hershey, Pennsylvania 17033

AJIT KUMAR

Department of Microbiology and Molecular Genetics,
Harvard Medical School, Surgical Services,
Massachusetts General Hospital,
Shriners Burns Institute,
Boston, Massachusetts 02114

Received 21 December 1978; accepted 21 March 1979.

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A reversible conformational transition in chromatin

THE DNA in the nucleosome is tightly wound in about two turns around a core protein octamer (reviewed in refs 1 and 2), and in this conformation the two strands of the double helix cannot be separated. Chromatin known to be transcriptionally active exhibits differences, both in the packing ratio of the DNA and in appearance under the electron microscope. At the same time, the periodicity revealed by nuclease digestion of nucleosomes is preserved, and histones remain attached to the DNA^{2,3}. This evidence strongly suggests that chromatin has the ability to undergo a conformational transition between a folded and an open state. Previous electron microscopic observations^{4,5} have revealed that at very low ionic strengths nucleosomes undergo two transitions, one apparently involving a dissociation into 'half-nucleosomes', the other a transformation to a fully extended state. Transitions have also been deduced from hydrodynamic studies⁶ and analysis of thermal denaturation profiles^{7,8}. All these methods are applied to dilute solutions and are confined to conditions in which the chromatin is soluble. On the other hand, methods usable at high concentrations have failed to produce evidence of an unfolded state at neutral pH, even when the concentrated chromatin solution was first dialysed against solutions of very low ionic strength. This seeming inconsistency can be understood, however, if one considers the concentration of the excess negative charges on the phosphate groups of the DNA in the condensed state, for as a consequence of the Donnan effect, one cannot reduce the sodium ion concentration below the level of charge equivalence without also lowering the pH. We now report the characterisation of a pH-dependent reversible transition of chromatin to an unfolded form.

We set out first to establish whether the unfolding transition of chromatin, apparently observed at low salt concentrations, could be reproduced in X-ray diffraction experiments with highly concentrated samples. To replace the sodium ions by protons we dialysed a 20% chromatin gel against distilled water containing an equivalent amount of Dowex 50W-X8 in the hydrogen form, in suspension. When the pH fell below 5 the chromatin precipitated, but when the precipitate was washed in water titrated to pH 5.0, its volume increased and it regained its gelatinous appearance.

Figure 1 illustrates diffraction from the two states. Both patterns show a broad 1.2-nm maximum (not illustrated), indicating that the double-helical structure of DNA is preserved. Unfolding is indicated by the disappearance of the characteristic packing and internal-structure peaks (restored in the second pattern) at about 11, 5.5 and 3.7 nm. The 'unfolded'

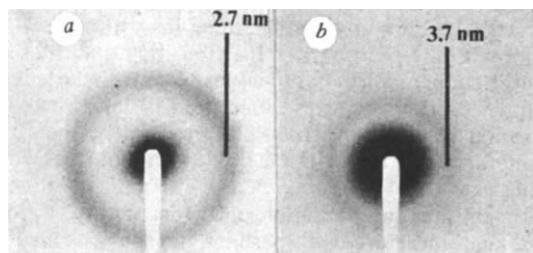


Fig. 1 X-ray diffraction pattern of chromatin: *a*, dialysed against distilled water with an equivalent amount of Dowex 50W-X8 in hydrogen form and *b*, washed after that in water titrated to pH 5.0. Diffraction patterns were obtained on a Franks camera of specimen-to-film distance 85 mm, using a rotating anode X-ray generator. Exposure times were about 36 h. Chromatin was obtained from Triton-prepared nuclei by washing in 25 mM EDTA, followed by swelling in water²⁶.

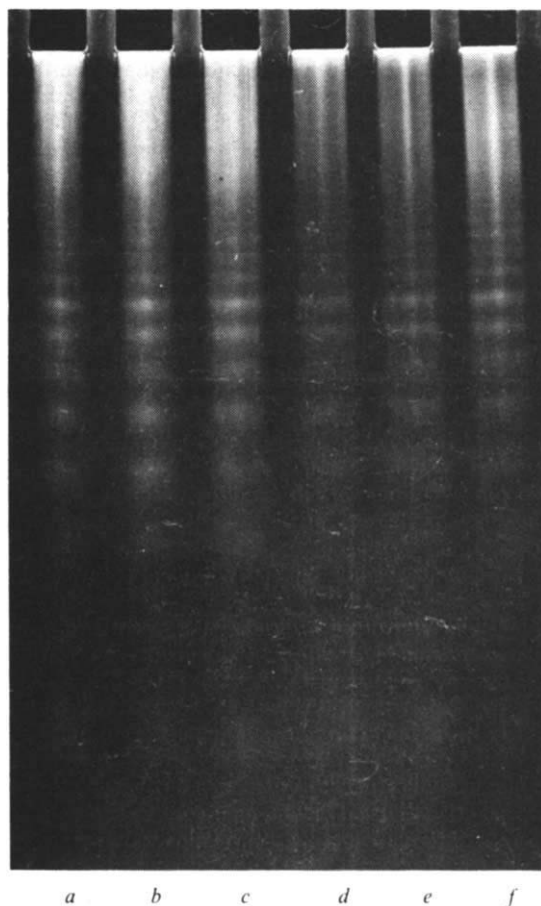


Fig. 2 Comparison of DNase I digests of acid-treated and acid-untreated chromatin. Chromatin prepared as described in Fig. 1 was acid treated by transferring 2 ml of chromatin (1 mg ml^{-1}) into a large volume (20 ml) of 20 mM sodium acetate, pH 4, and left overnight at 4 °C. The resulting precipitate was then transferred into a large volume of 1 mM Tris-HCl pH 7.5 and left to swell overnight. The gel was then transferred to 5 ml of the same buffer and swelling continued for a further 48 h. The gel was recovered by centrifugation. Untreated samples were stored at 4 °C for the same period. All buffers used for chromatin preparation and treatment contained 0.1 mM phenylmethylsulphonyl fluoride. Both chromatin, at 1 mg ml^{-1} , were digested with DNase I (Worthington) at an enzyme to substrate ratio of 60 units per mg chromatin in 6 mM CaCl_2 , 6 mM MgCl_2 , 10 mM Tris-HCl pH 7.5, at 37 °C. Aliquots were removed from the reaction mixture at intervals and the digestion was stopped by the addition of EDTA to a final concentration of 20 mM. DNA was extracted with phenol and chloroform and precipitated with ethanol before analysis in a denaturing, 10% polyacrylamide gel containing 7 M urea, 40 mM Tris-acetate, 20 mM sodium acetate, 2 mM EDTA pH 7.8. Before application to the gel, the DNA was denatured in 50% formamide by heating at 70 °C for 5 min. Gels were stained with ethidium bromide and photographed under UV illumination. Increasing times of digestion (0.5, 1 and 2 min) are shown from left to right. Untreated chromatin (*a*, *b* and *c*); treated chromatin (*d*, *e* and *f*).

pattern shows instead a strong, isolated maximum at 2.7–3.0 nm. The 11-nm peak is an interparticle interference maximum, representing either an internucleosome contact distance or the average separation of particles in a liquid-type array of mutually repulsive nucleosomes^{9–13}. The 5.5-nm peak may also be due mainly to an interparticle effect arising from the stacking of nucleosomes in columns parallel to their short axes^{14,15}. The 3.7-nm maximum almost certainly arises from the structure of the individual nucleosome^{16,17}.

The disappearance of all these peaks is therefore a clear indication that the internal structure and packing of the nucleosome have been disrupted. The new peak at about 2.7 nm is consistent with a new interparticle effect arising from the random close-packing of long cylinders with a mass per unit

length equal to that of extended DNA to which histones have remained attached^{9,18}. Such a diffraction maximum has been observed many times before, either on its own¹⁸⁻²⁰ or mixed with the 'native' pattern^{18,21}, but the conditions for its appearance in solution have never been defined. It has sometimes been attributed to the internal structure of the nucleosome, but a study of all the available data⁹ suggests that this may be a mistake; at high chromatin concentrations the peak varies widely in intensity from one preparation to another. On the other hand there is good evidence that it arises largely from the DNA component^{10,22}, in agreement with the model for an unfolded structure, in which DNA molecules are hexagonally packed. No globular structure comparable in size with a nucleosome could give an interparticle peak at a spacing as small as 2.7 nm.

There were early reports of a transition to this 'unfolded' state, induced by stretching a fibre^{23,24} or film²¹ of chromatin. In the first case the transition was reversible. What is new is our ability to induce the process in solution by a change of pH, and to restore the native structure reversibly. This should open the way to a number of useful biochemical experiments. The effect of pH on nucleosomes has been studied by Zama *et al.*²⁵, who

described a spectroscopic transition in mononucleosomes over the same pH interval as the structural changes described above. However, their transition was stated to be irreversible and precipitation occurred at about pH 4. We suggest that this precipitation (which does not interfere with X-ray diffraction) is due to the exposure of hydrophobic regions in the unfolded nucleosome.

Most diffraction work has been done on samples that were not buffered or were kept at a very low ionic strength. At the same time the chromatin concentration was often very high. In view of our finding that a fairly modest change of pH, probably affecting only some histone carboxyl groups, can completely unfold a sample, it is reasonable to suggest that in many past experiments at least a proportion of the material had undergone the transition. A stable component diffracting at 2.7 nm would then persist, as observed, and small aggregates of this kind could well escape visual notice in a highly concentrated gel. Some of the changes attributed to shear may really have been due to variation of pH. The modest energy barrier to the transition also leads us to believe that low-pH structure has biological relevance; the transition might be induced *in vivo* by histone modification or by some natural factor that remains to be identified.

The cycle of transformations illustrated in Fig. 1 has also been reproduced in a buffered system using 25 and 50% chromatin gels. The transition occurred over a pH interval of less than 0.5 units, beginning at about pH 4.35, and ending at pH 4.0. This transition was observed in 20 mM acetate even in the presence of sodium chloride up to a concentration of 40 mM. However, if 80 mM sodium chloride was present when the pH was lowered, the ensuing precipitate, although similar in appearance to that formed at lower salt concentration, gave diffraction showing that only a small proportion had unfolded; when left for 11 days in the same medium, very little further change occurred. This stabilisation of the structure by salt applied also to the reverse change. Thus, whereas overnight re-equilibration of the unfolded precipitated chromatin with 1 mM Tris pH 7.5 led to recovery of the original X-ray pattern, no such reversal occurred when 80 mM sodium chloride was also present, even after many days.

To demonstrate that the recovery of the native X-ray diffraction pattern is indeed associated with the return of the original structure, with its characteristic internal periodicities intact, digestion experiments were performed. As shown in Fig. 2 the series of fragments generated by digestion with DNase I is identical for the original material and for that restored to neutral pH after treatment at pH 4. It follows from this that the nucleosome structure is recovered. Digestion with micrococcal nuclease (Fig. 3) also shows that the distribution of nucleosomes along the DNA helix is unchanged, that is, no 'sliding' has occurred. We have not so far been able to obtain digests of material in the unfolded state because of the technical problems of digesting a precipitate.

The mechanism of the pH-induced structural transitions is still uncertain. The relative lack of dependence of the pH profile of the structural change on the ionic strength, up to at least 40 mM, together with the sharpness of the transition, indicates a high degree of cooperativity. One may ask whether the effect is a consequence primarily of the ionisation of DNA bases or of protonation of histone carboxylate groups. The ionisation of cytosine and adenine in DNA sets in at about pH 3.8, and becomes extensive by pH 2.8 (ref. 25). This makes it more likely at first sight that the ionisation of histone carboxyl groups is critical, and therefore that perhaps intranucleosomal interactions between carboxylate and lysine or arginine side chains may be a major determinant of the structural state. At higher salt concentrations the two conformational states may be 'frozen' and interconversion inhibited. The elevated kinetic barrier to this process could be related to stabilisation of either structure by way of aggregation.

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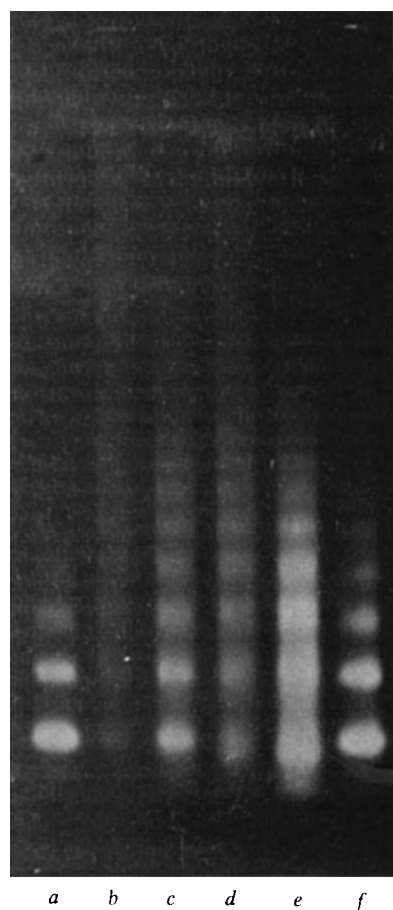


Fig. 3 Comparison of micrococcal nuclease digestion patterns of acid-treated and -untreated chromatin. Treated and untreated chromatin, prepared as described in Fig. 2, were digested, at a concentration of 1 mg ml^{-1} , with micrococcal nuclease at an enzyme-to-substrate ratio of 31 units per mg chromatin in 0.75 mM CaCl_2 , 10 mM Tris-HCl pH 7.5, at 37°C . Aliquots were removed from the reaction mixture at intervals and the digestion was stopped by the addition of EDTA to a final concentration of 2 mM . DNA was extracted with phenol and chloroform and precipitated with ethanol before analysis in a 1% agarose gel containing 30 mM Tris-HCl , $36 \text{ mM NaH}_2\text{PO}_4$, 1 mM EDTA pH 7.5. Gels were stained and photographed as described in Fig. 2. Increasing times of digestion (5 and 10 min) are shown from left to right. Untreated chromatin (b and c); treated chromatin (d and e). A chromatin standard digest prepared in a separate experiment is shown in slots a and f.

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Department of Biophysics,
King's College,
26-29 Drury Lane, London WC2, UK

D. Z. STAYNOV*
M. SPENCER
J. ALLAN
H. J. GOULD

Received 7 February; accepted 26 March 1979.

* Permanent address: Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

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Lobster shell carotenoprotein organisation *in situ* studied by photoacoustic spectroscopy

A SINGLE carotenoid pigment, astaxanthin, bound to specific proteins in a complex quaternary structure, provides shell coloration in the lobster *Homarus americanus*¹⁻⁸. The absorption of light by these carotenoproteins is uniform over a remarkable spectral range, as evidenced by the reflectance of a blue shell fragment which is low (5-6%) and essentially constant from 400 to 700 nm (ref. 9). This uniform absorption cannot be assigned to any combination of the carotenoprotein complexes which have been isolated. To assess the significance of these chemical species and to make specific inferences about carotenoprotein function *in vivo*, it is essential to measure the absorption spectrum *in situ*¹⁰. Several astaxanthin-protein complexes which absorb in the visible region have been isolated and spectroscopically characterised^{1-9,11}. These include α - and γ -crustacyanin with absorption maxima at 632 and 625 nm, respectively, and a second complex called the yellow protein^{7,11} which absorbs maximally at 410 nm. An early *in situ* absorption measurement of a carapace sample taken immediately after ecdysis and before calcification has been reported by Cheesman et al.⁴. However, the absence of structure in the reflectance measurements⁹ from a mature, opaque carapace suggests that conventional spectroscopy can yield little information about absorption maxima *in situ*. By using a new spectroscopic technique, photoacoustic spectroscopy (PAS), we have obtained spectra of a native shell fragment and here report the existence of a near continuum of astaxanthin absorption energies which span the visible spectral region. Furthermore, these pigments are in an anisotropic distribution within the endocuticle, that is, those absorbing in the blue are near the surface whereas those absorbing in the red are in the interior.

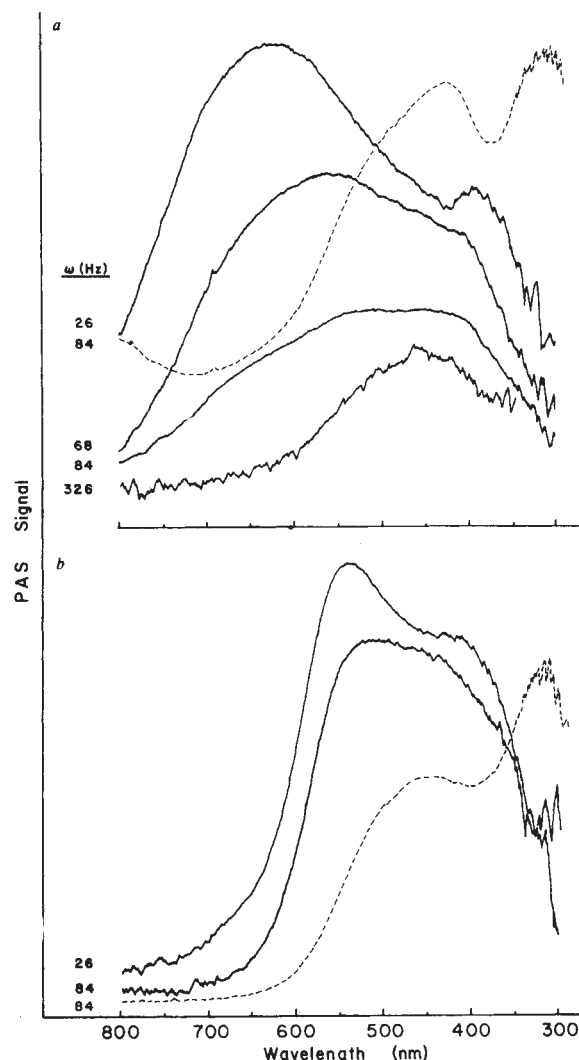


Fig. 1 Photoacoustic absorption spectra of native (a) and denatured (b) lobster shell as a function of modulation frequency ω (indicated with each spectrum) and phase ϕ . The interior spectra (—) were recorded with ϕ selected for maximum discrimination against the surface signal. In this case, decreasing ω allows response from deeper within the sample. The surface region spectra (---) were recorded with ϕ selected for maximum response from the surface. This selection of phase angle restricts the response to the surface region and results in a spectrum which is essentially independent of ω . Square wave modulation used in our spectrometer results in a triangular waveform from a carbon black (soot) sample and a concomitant 90° phase shift from the reference. Thus, the surface spectrum is optimised at $\phi = 90^\circ$ and discriminated against at $\phi = 0^\circ$. These spectra were recorded with a 10 nm spectral bandpass and a 0.03 Hz bandwidth (4-s time constant) at the lock-in output. The signal intensities are only qualitative.

PAS is ideally suited for measuring absorption maxima of opaque materials, and as it depends on thermal as well as optical properties of the sample, provides different information from reflectance measurements. It involves the measurement of heat produced as an excited species relaxes by a nonradiative path¹²⁻¹⁸. The exciting light is chopped at a suitable frequency and the resulting modulated heat flow is detected as pressure fluctuations using a microphone and a lock-in amplifier. As the exciting light is scanned, a spectrum similar to the absorption spectrum is obtained in which the response is proportional to both the absorption cross section and the thermal diffusivity of the sample. It is convenient to specify a thermal diffusion length¹³, $\mu = (2\alpha/\omega)^{1/2}$, where α is the thermal diffusivity and ω the chopping frequency. The definition of μ is such that light which penetrates the sample deeper than μ before absorption does not contribute to the response, as the periodicity is damped out

before the heat diffuses to the surface. Additionally, the phase (ϕ) of the signal at the phase-sensitive detector is critically dependent on the thermal diffusion time, as phase shifts are induced by the time required for thermal diffusion to the sample surface¹⁵⁻¹⁷. Spectral band shapes are influenced by several factors including wavelength-dependent relaxation pathways, saturation effects¹⁴ and, in layered samples, the filter effect of pigments located near the surface. Nevertheless, band maxima from PAS agree very well with measurements by conventional absorption techniques, and the spectrum is independent of ω and ϕ for an isotropic sample, provided saturation is avoided¹⁴. A key feature of PAS is that both ϕ and μ are experimental parameters that can be chosen to optimise the response from different levels of the sample^{16,17}. In certain cases the pigment locations in anisotropic (layered) samples can be determined¹⁵.

The spectra presented here are of a small sample ($\sim 4 \text{ mm}^2$) of uniform deep blue-green colour which was removed from the carapace of a live, mature lobster and used without further preparation. Samples removed from regions of carapace of similar appearance yield indistinguishable results, and samples from regions of obviously different coloration, such as the yellow underside of the claw, yield distinctively different spectra.

Figure 1a presents a series of spectra obtained at different modulation frequencies and phases. It is immediately apparent that the pigment distribution in the carapace is anisotropic so that by changing ω and ϕ one gains access to the different classes (layers) of pigments. Beginning with the surface region, absorption in the near UV by the epicuticle (which consists of wax, polyphenols and various lipoproteins^{4,19}) is evident, together with a band at $\sim 425 \text{ nm}$ which is assigned to the outermost carotenoprotein in the endocuticle. The spectra from the interior of the endocuticle demonstrate a remarkable bathochromic shift in the carotenoprotein absorption, from ~ 450 to $\sim 625 \text{ nm}$, as the modulation frequency is lowered from 326 to 26 Hz. The exception to this trend is the band at 390 nm which is clearly evident in the 26 Hz spectrum and not present in the spectrum from the surface region.

Figure 1b presents the results of a similar study on the shell fragment after denaturation of the carotenoproteins by boiling. The surface region spectrum shows that although the absorption of the epicuticle remains unchanged, the carotenoprotein band at $\sim 425 \text{ nm}$ is shifted to $\sim 450 \text{ nm}$. Only a small change in the interior spectrum is observed as ω is changed. The absorption in the red has disappeared and there is a general appearance of absorption strength in the region 450–560 nm.

We propose that the progressive bathochromic shift of the pigment absorption as a function of depth in the native endocuticle is due to highly specific protein-protein, pigment-protein and pigment-pigment interactions. If this is correct, then it is expected that denaturation would tend to convert all the chromophores to a common form. Indeed, this is the case, and thus the observation of the expected spectra from the denatured sample strongly supports the assignment of an anisotropic distribution of bathochromically shifted pigments in the native endocuticle.

The assignment of bands observed *in situ* to crustacyanins which have been isolated and purified is not straightforward. The spectra presented here, as well as the reflectance spectrum, are characteristic of an essentially continuous shift in the absorption maximum of the crustacyanins. This shift is produced by the interactions described above, which are expected to be a part of the ultrastructure of the endocuticle. Thus, it may not be possible to isolate discrete chemical species corresponding to each absorption. In the case of the denatured shell, the range of absorption maxima between 450 and 560 nm is characteristic of β -crustacyanin and other products of the denaturation of α -crustacyanin^{2,7,8}.

Astaxanthin aggregates, which have been described in terms of exciton states^{11,20}, absorb maximally at 390 nm. This corresponds exactly to the 390 nm band in the native shell and suggests the assignment of this band to the yellow protein *in situ* in which exciton interactions have also been found, although

after isolation and purification, absorption shifts to 410 nm (refs 2,7,9). Assuming a simple exciton model^{21,22} for this species, a monomer absorption maximum at 490 nm and the allowed exciton state at 390 nm, one calculates the corresponding bathochromically shifted and less allowed exciton state to be at 660 nm. Indeed, the absorption spectrum of purified yellow protein² clearly shows absorption by the less allowed transition in this region. Thus, the anisotropic distribution of bathochromically shifted excited states is such that the lowest energy level, the bathochromically shifted *exciton* state, is in the interior of the endocuticle. This assignment of an interior location to the yellow protein is in contrast to the recent suggestion based on resonance Raman scattering that it is at the shell surface⁹.

A quantitative description of the location of the various carotenoproteins may be obtained from measurements of the phase shift as a function of ω , provided α is known^{16,17}. In the present study, a qualitative estimation of depth is given by μ which ranges from 10 μm at 326 Hz to 35 μm at 26 Hz, if α is taken as $0.001 \text{ cm}^2 \text{ s}^{-1}$. This value of α is typical of biological materials²³ similar to chitin and yields values of μ in excellent agreement with the observed thickness of the epicuticle and depth of the pigmented zone¹⁹.

Although the work described here is structural in that it reports only the detection of a variety of carotenoprotein species and their organisation in the lobster shell, several interpretations are possible. The anisotropic arrangement of the pigments may not serve any photobiological role and may simply be a consequence of the ordered synthesis of the pigmented zone during moulting. Functions such as cryptic coloration may or may not be a property of the distribution of the pigments in layers. However, two possible functions which are dependent on pigment arrangement may be suggested. First, with different receptors serving specific layers of pigment a form of colour discrimination is obtained which could be important in a variety of photobiological processes. Second, pigments arranged with the lowest excited state in the interior could function as an antenna which absorbs light throughout the visible range and transfers excitation energy vectorially to the interior of the endocuticle where receptor sites may be located.

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Department of Chemistry,
Arizona State University,
Tempe, Arizona 85281

MICHAEL L. MACKENTHUN
RODERICK D. TOM
THOMAS A. MOORE*

Received 28 December 1978; accepted 7 March 1979.

*To whom correspondence should be addressed.

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reviews

Energy policy in the West

Gerald Foley

Western Energy Policy: The Case for Competition. By D. Evans. Pp. 198. (Macmillan: London, 1979.) £10.

THE title might suggest at least a stimulating polemic. Western energy policy, if it can be said to exist as an entity, is a mess. Uneconomic coal production competes for a scarcely growing electricity market with increasingly questioned nuclear power; both, in consequence, find themselves needing to be subsidised. Oil prices are set by the mood of the OPEC cartel rather than production costs. Natural gas, priced far below its replacement costs, threatens to drive its competitors, and possible successors, out of the domestic and prime industrial markets. Policy is an improvised assembly of measures to deal with an inheritance of inertia, legislation, established interests, and social constraints rather than a strategy deliberately designed to take industrial society through its short-term difficulties on to a path to an attainable and tolerable future.

At such times the clarity of the classical market theory becomes irresistibly appealing to those who feel that even complicated questions should have simple answers. Douglas Evans is one who believes that "it has been one of the sadder features of the post-war British economy to be more concerned with the distribution of income rather than the utilisation of resources", a condition brought about by governments which have "eroded the characteristic qualities of free competition". His work "makes no secret of its belief in the virtues of a free enterprise framework".

The book, however, totally fails to make any coherent case for a free market in energy. In fact, it is, in the end, difficult to know what case is being made at all. It provides a brief and superficial survey of past energy trends in the EEC countries, Japan, the US, and even makes a brief nod in the direction of China. There follows a look at some of the present energy policies, and objectives, of the UK, Germany, and the US. The vicissitudes of President Carter's energy proposals, which have still not reached an end, are described at length. One must sympathise with anyone trying to draw all the activity—and non-activity—in energy policy making in the Western

nations into a coherent pattern: it is a very difficult task. But because it does not provide a clearly defined viewpoint or establish a coherent set of evaluative criteria—apart from stating a few truisms such as the desirability of using energy more efficiently, and the need to ensure policies are humane and effective—Mr Evans' analysis is far more in the tradition of anecdotal newspaper journalism than a serious critique of policy. Having read it one is somewhat better informed on contemporary energy matters but is still left without any real feel for the strength of the forces at work or for the magnitude and nature of the problems to be solved.

There are also errors and misspellings about which one should not become pedantic; nevertheless, they have an undermining effect. Liquefied natural gas is not the same as synthetic natural gas (SNG). Britain does not export 40 million tons of coal now to the EEC, or anywhere else. The size of power stations is usually given in megawatts—not milowatts. And surely it is not true that "because they invariably entail an extended and often complex

process of decentralisation, conservation policies take some years to prove their effectiveness, that is, their lead times are long—to use the jargon of energy planners". There is a mountain of evidence to the contrary. Simple and inexpensive conservation measures such as draught-stripping, pipe-lagging, improved maintenance of boiler systems, cavity wall insulation and installation of control systems, can provide almost instant and, at times, quite dramatic savings in energy consumption.

This is not a book completely without merit. It puts together a lot of information about energy policy in the Western industrial world; it shows, by that alone, how much work needs to be done. And Evans is often shrewd and perceptive in his comments. But the book he has written does nothing to make a case for founding future policies on increasing competition between energy suppliers. Neither does it offer any guidance to what might be done instead. □

Gerald Foley is a Fellow of the International Institute for Environment and Development (IIED), London, UK.

Drops, bubbles and solid particles

Bubbles, Drops, and Particles. By R. Clift, J. R. Grace and M. E. Weber. Pp. 380. (Academic: New York, San Francisco and London, 1978.) \$32; £20.80.

THE literature on the hydrodynamics of drops, bubbles and solid particles is notorious for the enormous range of journals in which important contributions appear. Thus, a book which attains the author's objective of presenting a "comprehensive, critical review of the literature" would be of great value.

In the area in which this reviewer has specialised knowledge, few references are omitted so that the claim to be comprehensive can be supported. The reader can thus work his way into the literature of a particular problem aided by a clear summary of the principal papers he needs to read. This in itself would make the book worthwhile. However, a work of length many times the 380 pages at the authors'

disposal would be required for a really deep critical account, so the authors' second claim is perhaps less justified. Nevertheless, the text is, considering the vast amount of information it carries, readable, and it manages to point up areas where more work is needed. In discussing the effect of surfactants on bubble drag at large Reynolds numbers (p 135), for example, the authors note that "accurate experimental data with known surfactant concentrations do not appear to be available" and "the conditions which must be satisfied for the theories to hold are so stringent that [the] theories are of little practical importance"; a challenge to both experimenters and theoreticians.

The organisation of the book is excellent. It was an inspired thought to place the numbers of the chapters in which various shape regimes are studied on a Reynolds number versus Eotvos number diagram, so that the plan of the work can be absorbed at a glance. In

chapters 3 to 8 the flow problem is first described before the associated forced convection problem—which arises when the drop, bubble or particle has a concentration not equal to that of the continuous phase—is discussed. This systematic plan enables the reader easily to find his way about in individual chapters.

The last four chapters cover wall effects, shear and accelerated flow and formation and break-up of fluid particles, necessarily rather briefly.

This reviewer has no doubt of the

value of the book to chemical engineers and others with a special interest in drops, bubbles and particles. However, it is to be hoped that it will persuade theoretical hydrodynamicists to take an interest in some of the fascinating unsolved problems, particularly in the 'skirted' and 'dimpled' cap bubbles described in chapter 8.

D. W. Moore

D. W. Moore is Professor of Applied Mathematics at Imperial College, University of London, UK.

Ionosphere phenomena

Non-Linear Phenomena in the Ionosphere. By A. V. Gurevich. (Springer: New York, Heidelberg and Berlin, 1978.) DM98; \$49.

THIS monograph is the tenth in the series published by Springer under the general title *Physics and Chemistry in Space*, which is intended "to provide student teachers and researchers with clear and concise presentations of up-to-date topics in space phenomena". Each of the volumes is prepared by a recognised international authority in the field, and this latest text is a very worthy addition to the series.

In April 1933, during the early years of ground-based radio wave studies of the ionosphere, Tellegen observed that radio signals received at Eindhoven, Holland, on 460 metres from the Swiss station Beromünster were modulated by signals from the newly established high-power Luxembourg station operating on 1,190 metres. It was soon established that the phenomenon was not due to cross-modulation within the receiving equipment, and in 1934 V. A. Bailey and D. F. Martyn showed theoretically how this so-called 'Luxembourg effect' could be explained in terms of non-linear interaction of the two radio signals in the lower part of the ionosphere. In the years immediately following World War II the theory of the phenomena became more fully worked out and wave-interaction was developed as an additional diagnostic tool for studying the lower levels of the ionosphere. In this period cross-modulation resonance effects of gyrofrequencies were also observed, and the possibility of ionospheric 'self-demodulation' discussed theoretically and observed experimentally. In recent years the availability of radio transmitters radiating power large enough to perturb markedly the lower ionosphere and to modify the ionosphere at higher levels, has resulted in a greatly renewed interest by both theoreticians and experimentalists in many other aspects of ionospheric wave interaction pheno-

mena. The variety of ionospheric modifications successfully effected is now quite extensive and includes large-scale stratifications leading to the creation of an artificial 'sporadic F' layer, the formation of inhomogeneities elongated along the magnetic field, the excitation of plasma and ion-acoustic waves, luminescence produced by accelerated electrons (a possibility first considered

by V. A. Bailey in 1937), and the precipitation of fast electrons from the plasmasphere.

For more than two decades major contributions to the theory of these various non-linear phenomena have been made by Dr A. V. Gurevich, and the clarity and conciseness of this volume is a testimony to his profound understanding of the subject.

Apart from one or two very minor matters, the translation is excellent and the standard of printing and production of the volume are both of the very highest quality. The monograph is well indexed, includes a comprehensive list of references, and can be strongly recommended to all concerned with this very topical aspect of ionospheric physics.

W. J. Granville Beynon

Sir Granville Beynon is Professor and Head of the Department of Physics at the University College of Wales, Aberystwyth, UK.

Group theory and nuclear symmetries

Group Symmetries in Nuclear Structure. By J. C. Parikh. Pp. 277. (Plenum: New York and London, 1978.) \$40.20.

THE structure of the atomic nucleus presents a demanding and complex problem to the physicist, with ramifications which touch on many areas of fundamental research. As with atomic structure, the source of the complexity is that it is a problem with many particles in interaction, but not so very many that the change from n particles to $n+1$ is not significant—indeed, those changes are amongst the most interesting features of the problem. But in atomic physics one has the advantage that the fundamental interaction between the particles involved is known and simple; it is the electromagnetic interaction. The nuclear structure physicist must cope with the complicated, largely empirically determined form of the nuclear forces. Of enormous value in bringing order to this complexity is the use of group theory to exploit the symmetries, exact and approximate, fundamental and phenomenological, which nuclei possess.

This monograph is concerned with presenting these symmetries, especially the dynamical symmetries which arise in the finite-dimensional state-space of approximation schemes like the shell model, and at the same time with the introduction of the basic mathematical ideas and techniques of group theory.

The level of the treatment is appropriate to postgraduate students and research workers. It is intended to be useful to both experimentalists and theorists, but I doubt if any but the most dedicated experimentalists would in fact wish to tackle the book in its entirety. It is a specialised monograph, a review rather than a textbook. To my mind it would have been more useful if it had placed the specific topics covered in a broader context. The nuclear physics is used to illustrate methods and problems in group theory rather than the other way round, and in my view that is a weakness. However, there are good introductions to the important topics of seniority and symplectic symmetry, of Wigner's SU(4) and Elliott's SU(3), and of their extensions. An interesting treatment of a method due to French for deriving good approximations to binding energies, spectra, and so on, by using moments of the spectral distribution of the Hamiltonian, forms a central feature of the monograph.

For the nuclear structure theorist who wants a detailed study of the use and application of group theory in this field, I know of no other recent book. Although the work originated in a lecture course given in 1970, some more recent work is cited; it's a pity, however, that the latest reference brings us no further than to 1975.

In summary, this is a competent but specialist treatment for the specialist worker in the field.

John M. Charap

John M. Charap is Professor of Theoretical Physics at Queen Mary College, University of London, UK.

Pesticides and microorganisms

Pesticide Microbiology: Microbiological Aspects of Pesticide Behaviour in the Environment. Edited by I. R. Hill and S. J. L. Wright. Pp. 844. (Academic: London, New York and San Francisco, 1978.) £38; \$78.65.

'SAFETY' is the current climate and there are stringent conditions governing the registration of pesticides, which are by their nature, biologically very active. These include microbiological investigations. This book is an attempt, and a very successful one, to bring together much of the literature on the reactions that take place between microorganisms (bacteria, fungi, microalgae and microfauna) in the various habitats (soil, air, water, plants and animals) in which they are found, and the various pesticides (insecticides, herbicides, fungicides and soil fumigants) which are used in agriculture and public health. This is a field (as indeed are all pesticide investigations) in which academic and industrial scientists meet on common ground with much benefit to both and this happy cooperation (which your reviewer has experienced) is reflected in the make-up of this book. One of the authors (I. R. Hill) is from ICI and

the other (S. J. L. Wright) from the University of Bath. Their interests are reflected in the choice of authors—five from industry, five from universities, and three from research institutes. The result is a happy blending of fundamental and applied aspects.

The editors have done a splendid job in bringing together specialists from the various fields, and although they have obviously had difficulty in persuading all of them to meet publishing deadlines (a common hazard!) the finished products are works of real scholarship. Pesticide microbiology has really come of age academically and this volume (in the various editions that will surely follow) should be the standard text for workers in the field for many years to come. One of the most pleasing features of the book, at least to this reviewer, is that each chapter finishes with a set of "Con-

clusions". There are so many investigations using such a variety of techniques that the reader is in danger of becoming bewildered and lost in a mass of data. But don't expect the conclusions to be conclusive. The environment is too variable a medium for that, but a little drawing together of information is welcome.

This is essentially a book for the research worker, and in this respect one might question the value of chapters one and two, which are introductory in nature. Although both chapters are well written their exclusion would have thinned the book a little and might have reduced the rather frightening price of £38.

W. W. Fletcher

W. W. Fletcher is Professor of Biology at the University of Strathclyde, Glasgow, UK.

Processes of osmoregulation

Mechanisms of Osmoregulation in Animals: Maintenance of Cell Volume. Edited by R. Gilles. Pp. 667. (Wiley: Chichester, UK, and New York, 1979.) £30.

ONE of the vital activities of animals, and ultimately of cells, is an ability to regulate their water and solute content. This process, which results in the maintenance of the osmotic pressure and volume of cells, can take place in the face of a wide variety of environmental conditions which may be associated with many modifications in morphology and physiology. The occupation by animals of such contrasting habitats as the oceans, lakes of fresh-water and hot arid deserts is only made possible as a result of such adaptations. Claude Bernard more than 100 years ago elevated the study of this subject to one suitable for intellectual reflection and he also, to use a modern idiom, made it 'relevant'. Osmoregulation has since attracted people from many disciplines including biology, medicine, and even the physical and environmental sciences.

For this book Dr Gilles has assembled a group of authors which reflects this widespread interest in osmoregulation and provides the reader with an excellent opportunity to benefit from the expertise of each. The survey is broad in its design, including both vertebrates and invertebrates and it ranges from basic physicochemical principles, as applied to cells, to the nature of some pathological disorders of fluid regulation in man. I found the basic mechanisms of osmoregulation to be especially well described throughout the book and

this should provide a very useful basis for teaching students. A comparative survey of osmoregulation among the vertebrates has been provided, which is well presented; but presumably because of limitations on the size of the book it is not a complete one. Apart from their kidneys, mammals have been relatively neglected and some interesting and exotic groups such as the cyclostome fishes and lungfishes get little attention. The roles and mechanisms of action of hormones in osmoregulation of vertebrates have been admirably described. However, the interesting effects of prolactin, which helps regulate the secretion of milk in mammals but osmoregulation in some fishes, possibly merits more attention.

The contributions are up to date and include full references up to 1976 with some as recent as 1978. The text is clearly set out so that it is easy to find and select topics of interest. I enjoyed reading this book and have no hesitation in recommending it to graduate students and research scientists who are interested in this subject. It will be especially useful to those who are searching for a clear summary of background material on which to build a sound knowledge of the processes of osmoregulation. This volume also contains much of general interest for students in zoology and physiology. The price suggests that the book is mainly directed towards libraries so that unfortunately its accessibility to students may be limited. Possibly the publishers should consider a paperback edition for the impecunious?

Peter J. Bentley

Peter J. Bentley is Professor of Pharmacology at the Mount Sinai School of Medicine, Mount Sinai Hospital, New York.

EARTHQUAKES:

Cause, Prediction, and Control

J. H. Tatsch

We are pleased to report that this book is selling well in all parts of the world. The phenomenal demand for *Earthquakes* arises primarily from the fact that it explains how to determine where (and where not) to place nuclear power plants in order to minimize the possibility of earthquake damage.

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Disease caused by asbestos

Asbestos and Disease. By I. J. Selikoff and D. H. K. Lee. Pp. 549. (Academic: New York, San Francisco and London, 1978.) \$31.50; £20.45.

DR SELIKOFF and Dr Lee have attempted to provide a comprehensive review of disease caused by asbestos dust "to provide non-specialists with easily comprehensible and meaningful data". With help from many colleagues at the Mount Sinai School of Medicine, the text ranges widely over asbestos and its production, disease effects (asbestosis, cancer, pathogenic mechanisms), and prevention and control. "As individuals we naturally have come to our own conclusions but we have tried to present the evidence for and against the various interpretations so that readers can attempt their own interpretations if they wish."

This is a laudable aim, but in at least one most important aspect it does not seem to have been attempted. Asbestos may be divided into two quite distinct groups: the amphiboles and chrysotile. Indeed the excellent chapter on the nature, occurrence and properties of asbestos minerals considers the distinction in detail. Yet the epidemiological evidence for there being differing biological effects is largely ignored or skipped over. This epidemiological evidence was already extensive enough in 1970 for the UK asbestos regulations to be ten times stricter for crocidolite than for chrysotile. Selikoff and Lee comment that this is characteristic of the British evaluation—implying their disagreement—but they do not provide any contrary argument.

The treatment of the positive epidemiological evidence is somewhat brusque. Braun and Truan (*Arch. Ind. Health*, **17**, 634–653; 1958) are criticised because in their study of chrysotile miners there was only a 97.8% follow-up. The data of McDonald *et al.* (*Arch. Environ. Health*, **22**, 677–686; 1971; a long-term mortality study of 10,421 chrysotile miners and millers) are described as "meager". The evidence from asbestos cement workers (Weill *et al.* in *Inhaled Particles*, **4**, 789–798; Pergamon, 1977) exposed to crocidolite and chrysotile is not even discussed. In the four cohort studies by the Mount Sinai investigators, the fourth has the lowest mortality ratios for cancer of all sites and for pulmonary carcinoma (Table 12-1), yet the reader is not reminded that this is a chrysotile-exposed group until twenty pages on. Nor is the reader told that the asbestos miners and millers in the Urals (Kogan *et al.*, *Gig. Sanit.*, **37**,

29–32; 1972) with lower mortality ratios are chrysotile exposed. The size component of Timbrell's 1972 theory (in *Assessment of Airborne Particles*, 429–445; Charles C. Thomas, 1972) to account for differences based on aerodynamics of fibres was discussed. It is a pity that the shape component was not, as this accounts acceptably for observed differences.

It has been well known for many years that Dr Selikoff does not accept that crocidolite and chrysotile have different effects even in degree. Even though he was a member of the International Advisory Panel on Epidemiology to the Director of the International Agency for Research on Cancer in 1972 (IARC Publication 8; Lyon, 1973) he did not attend the panel meeting and he dissociated himself from the advisory report, which emphasised "that there are clear differences in risk with type of fibre". This book would have been an excellent opportunity to justify his opposing view.

The proof reading of the text was obviously very careful as your reviewer found only two spelling errors and

these were both on the first page. However, the unusual presentation of scales in Figure 10-4 has led to an error; the captions to plates 12-1d and e have been interchanged; several references in later chapters to Figures 10-6 and 10-7 should be to 10-4 and 10-5; and Merchant *et al.* (*Br. Med. J.*, **1**, 189–191, 1975; page 237) are wrongly quoted. A list of these and a few other errors has been sent to the authors and is available on request.

Presumably the power of the name of Selikoff will mean that most specialists in the USA will buy this book. However, the proceedings of the 1972 working conference on the *Biological Effects of Asbestos* (IARC Publication 8; Lyon, 1973) provide an equally extensive review and the proceedings of the 1979 working conference on the *Biological Effects of Mineral Fibres* (IARC: Lyon) will provide one more up-to-date.

Charles E. Rossiter

Charles E. Rossiter is a member of the scientific staff at the MRC Pneumoconiosis Unit, Penarth, UK.

Plant pathogenesis

Genetic and Molecular Basis of Plant Pathogenesis. By J. E. Vanderplank. (Springer: Berlin, Heidelberg and New York, 1978.) DM48; \$24.

THIS is the sixth volume in the Advanced Series in Agricultural Sciences and the third on plant pathology. The first of the three is reasonably idiosyncratic, the second more so. The third could have been written only by Dr Vanderplank, distinguished for his work on the epidemiology of plant diseases and well known for his provocative views on the population genetics of pathogens and their host plants, one of the two main subjects of the book. In his treatment of it in this book Dr Vanderplank uses freely the data of other scientists for analyses, comments and hypotheses. He emphasises that he is concerned almost wholly with diseases in which genes for resistance in the host are matched by genes for avirulence in the pathogen, especially blight of potato caused by *Phytophthora infestans*, and rusts of cereals caused by *Puccinia* spp. There are stimulating accounts of related and unrelated variation in disease resistance and pathogenicity, of the gene for gene hypothesis, of population genetics of the pathogen, and of horizontal resistance. Short chapters of seven pages deal with common antigens of host and pathogen, and host-selective toxins. Another ten pages is on the role in specificity of a wide range of large molecules such as DNA, RNA, glyco-

proteins, lectins and polysaccharides. These three chapters are, for the most part, non-controversial. They are very brief, considering the complexity of the subjects.

Most of the rest of the book is about an all-encompassing protein for protein hypothesis which aims to account in quite simple terms both for gene for gene relations between pathogens and their host plants in vertical resistance, and for horizontal resistance. Thus, the penultimate and summarising chapter states quite unequivocally "... Vertical resistance or susceptibility is determined by polymerisation [of proteins]. Horizontal resistance or susceptibility is determined by catalysis or the products of catalysis. The hypothesis is as simple as that and it fits the facts ...". simple as that and it fits the facts ...". the facts are quite another matter.

Both the exposition of the hypothesis and its derivation from the data selected for the purpose are very controversial and speculative and I doubt that they are well placed in a text such as this. But they make for interesting, if at times somewhat outraged reading, and, with the less provocative parts, a book to which I shall often return. However, most of it is for the experienced and the initiated and parts of it are for the somewhat sceptical who, if like me, would dearly like to be proved wrong.

R. K. S. Wood

R. K. S. Wood is Professor of Pathology at Imperial College, University of London, UK.

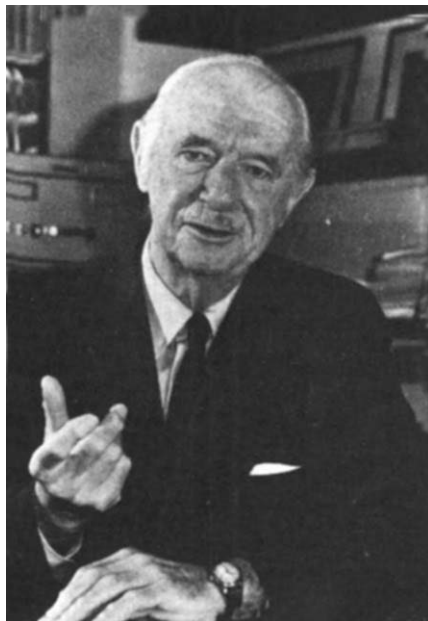
obituary

Vincent du Vigneaud, 1901-1978

VINCENT DU VIGNEAUD, Professor Emeritus of Biochemistry at Cornell University Medical College in New York City and, since 1967, Professor of Chemistry at Cornell University in Ithaca, New York, died, after a long illness, on 11 December 1978, in White Plains, New York.

Professor du Vigneaud, born in Chicago in 1901, was of French ancestry, the son of Alfred and Mary Theresa du Vigneaud. In 1918, he entered the University of Illinois in Urbana. Here he developed a sincere dedication to chemistry under the influence of C. S. Marvel, who taught him organic chemistry, H. B. Lewis, who lectured on cystine and cysteine, and last, but not least, W. C. Rose, who, in 1923, returning from a trip to Toronto, gave a talk on insulin, recently discovered there by Banting and Best. This lecture on the exciting new hormone made a lasting impression on the young student. In later years he liked to consider *sulphur* as the guiding principle of his research, leading him from insulin, through biotin and penicillin, to oxytocin and vasopressin (*A Trail of Research* by V. du Vigneaud, Cornell University Press, 1952). During the years in Urbana, he met Zella Zon Ford, an English major at the same University, his future wife and his constant source of encouragement until her death in 1977.

After graduation, he spent a short time in industry, in the laboratory of a hospital and taught chemistry in the School of Medicine at the University of Pennsylvania. Yet, the attraction of biochemistry was strong, and soon he joined Professor J. R. Murlin in the School of Medicine at the University of Rochester to work as a graduate student on the chemistry of insulin. This hormone was obtained in crystalline form by J. J. Abel, but the procedure was not readily reproducible and the young du Vigneaud demonstrated his exceptional experimental skills by preparing crystalline insulin in substantial quantity. Professor Murlin, himself a physiologist, gave him much freedom to pursue the origin of the alkali-labile sulphur in insulin, a study



which became his dissertation, *The Sulfur in Insulin*.

With a fellowship from the National Research Council, he joined Professor John J. Abel at The Johns Hopkins University Medical School. Here his insulin work was continued, in collaboration with Oscar Wintersteiner and Hans Jensen. The Fellowship was extended for a second year, which he spent in Germany at the Kaiser Wilhelm Institute in Dresden, in Max Bergmann's laboratory, where, working with Leonidas Zervas, he became involved in peptide synthesis. The studies abroad were concluded with shorter stays with Professor George Barger at the University of Edinburgh and with Dr Charles Harrington (later Sir Charles) at the University College Hospital Medical School in London.

Dr du Vigneaud returned to the United States to join the faculty of the Department of Physiology, headed by W. C. Rose, of the University of Illinois in Urbana, his alma mater. The stimulating atmosphere there is best shown by mentioning some of his colleagues: C. S. Marvel, R. C. Fuson, R. L. Shriner and last, but not least,

Roger Adams. Still, he yielded to the temptation of more independence in research and in 1932, at the age of 31, accepted an invitation from the George Washington University School of Medicine to head their Department of Biochemistry. Other invitations followed and in 1938 he made the decisive move, to New York City, to become Professor of Biochemistry and Head of the Department of Biochemistry at Cornell University Medical College, the scene of his greatest achievements. There du Vigneaud remained until his formal retirement in 1967 when he joined the Chemistry Department of Cornell at Ithaca, New York, as Professor of Chemistry. He carried out an active research programme for seven more years, when his creative work was ended by sudden illness.

It might be impractical to try even to sketch here du Vigneaud's contributions to the chemistry of insulin, his work on homocysteine and transmethylation, or on biotin. Let us point only to the leitmotif in these studies: they all contain sulphur. The surprising solubility of insulin in liquid ammonia prompted the development of the reductive cleavage of benzyl and benzyloxycarbonyl groups with sodium in liquid ammonia, a method which later turned out to be crucial in his studies on penicillin and on oxytocin.

The penicillin experiments were carried out during the Second World War as part of a concerted effort between forty cooperating laboratories. The Cornell group, under du Vigneaud, first accomplished a synthesis of penicillamine. The synthesis of penicillin itself followed. The structure on which the synthesis was based, the thiazolidine-azlactone formula is now considered erroneous (although Sir Robert Robinson showed that it is a 'protomer' of the finally accepted thiazolidine- β -lactam structure). Accordingly, the du Vigneaud synthesis resulted in an inactive product, but this could be readily converted to a material with slight, but definite antibiotic activity. The active component was demonstrated to be penicillin, by the isotope

dilution method and by its sensitivity to penicillinase.

At this point most researchers would have regarded the project as concluded. Not du Vigneaud. With his associates, he isolated penicillin G from the synthetic mixture through a series of purification steps, including the countercurrent distribution method developed about this time by Lyman C. Craig at the Rockefeller Institute, next door to Cornell. A crystalline salt of penicillin G was obtained and shown to be identical with natural penicillin by an array of methods of physical chemistry, X-ray crystallography and biological activity. This unrelenting and uncompromising search for conclusive evidence was a characteristic feature of the research of du Vigneaud. The same standards were maintained when his interest turned to the peptide hormones, oxytocin and vasopressin.

The hormonal effects of extracts from the posterior lobe of the pituitary gland have been known since the turn of the century. In the late nineteenth-twenties, O. Kamm demonstrated the presence of two separate principles in the extracts, one oxytocic (quickenning childbirth) and the other pressor and antidiuretic. A comment of Kamm on the relatively small molecular weight of the two hormonally active factors, oxytocin and vasopressin, sparked the interest of du Vigneaud. Although the penicillin studies interrupted his first attempts toward the isolation of the two peptide hormones in pure form, this effort was revived after the Second World War and led, in relatively short time, to the isolation of the pure peptides. Countercurrent distribution again played a major role in the success. Elucidation of the structures soon followed and in 1953 the endeavour was crowned by the synthesis of oxytocin, and later by the syntheses of lysine vasopressin and arginine vasopressin. These were epoch-making achievements. The 1955 Nobel Prize in Chemistry was awarded to Professor du Vigneaud 'for his work on biochemically important sulphur-containing compounds, especially for the first synthesis of a polypeptide hormone.' Other awards, medals, honorary degrees received by him are too numerous to be mentioned here.

Many of us thought that having finished with oxytocin, Professor du Vigneaud would turn towards even more ambitious objectives, perhaps the synthesis of insulin. On such questions his answer was: 'Finished? We have just started.' And, indeed, the effort in the field of pituitary hormones was continued for almost twenty more years. Many of the results from his laboratory were trend-setting, such as a new synthesis of oxytocin by the stepwise strategy, an approach which was

applied also to the synthesis of lysine vasopressin, and of numerous analogues of the two peptides. The availability of synthetic analogues permitted the exploration of the interaction between hormone and receptor. Functional groups were replaced by hydrogen, first the phenolic hydroxyl in oxytocin, then its single free amino group. Biologically active peptides were obtained. These studies gave new insight into hormonal activity and demonstrated the significance of architecture in peptides and proteins. In fact, Professor du Vigneaud liked to call oxytocin a 'baby protein.' The body of information generated in this tireless effort is impressive: it appeared in about 200 publications, almost half of the total 'output' of du Vigneaud's published work.

The published reports, however, are certainly not the total of the du Vigneaud legacy. The daily contact with this exceptional man had a major influence on those of us who were fortunate enough to have spent some of our formative years in his laboratory. His account of declining a suggestion to accept responsibility for the synthesis of corticotropin, which at that time seemed to be more important than oxytocin, taught us to stick to commitments already made. By insisting on treating synthetic oxytocin with bromine-water to observe its cleavage into two fragments, as happens with natural oxytocin, he demonstrated the need for skepticism towards one's own results, at a time when nobody else had doubts about their correctness.

On his prompting, I had prepared a crystalline salt of synthetic oxytocin suitable for X-ray studies. A letter to *Nature* on the crystals was already typed when he came to my laboratory with a sample of natural oxytocin and requested that I repeat the crystallisation with natural oxytocin as well. Years after receiving the Nobel Prize, he still wanted to be sure that there was no difference between natural and synthetic preparations of the hormone. Scientific integrity was one of the lessons we could learn from him. Yet, his style, his flair for the important and his unshakeable optimism that the next major discovery is just around the corner were unique aspects of the man, which would be difficult to imitate.

Professor du Vigneaud is survived by his two children, Vincent du Vigneaud, Jr., an obstetrician and gynaecologist, and Marilyn Renee Brown, Assistant Professor of Pediatrics at the University of Rochester Medical Center. Those who were saddened by his departure also include his former associates, the 166 members of the 'V. du V. Club,' among them many distinguished scientists, including two Nobel laureates.

Miklos Bodanszky

Robert van den Bosch

ROBERT VAN DEN BOSCH, Professor of Entomology and Chairman of the Division of Biological Control at the University of California, Berkeley, died while jogging on 19 November 1978. His death deprived the ranks of entomologists, ecologists, biological control specialists and environmentalists, world-wide, of an outstanding individual.

Van, as he was called by most who knew him, was born in Martinez, California on 31 March 1922. He received his BA in Physical Education from the University of California at Berkeley in 1943. From 1943 to 1946 he served as Deck Officer with the Amphibious Forces, US Naval Reserve, and saw heavy combat action in the Pacific. In 1947 he returned to the University of California, Berkeley as a student in entomology, receiving his PhD in that field in 1950.

In his professional career, besides being a researcher, a teacher, and an administrator in the Division of Biological Control and the Department of Entomological Sciences at the University of California, Berkeley, Van served as a member of the Board of Directors of the Rachel Carson Trust and a consultant to various national and international groups and institutions concerned with agricultural and environmental issues related to pest control. He was a fellow of the American Association for the Advancement of Sciences, and a Guggenheim Fellow.

The circuitous academic route followed by Professor van den Bosch was not as unusual as it might seem. In a communication written just before his death he clarified this by saying that bugs were his first love and that as a member of the Boy Scouts he was one of the rare ones who earned a merit badge in entomology. This was the major he chose when he came to the University of California as an undergraduate student in 1939. However, his interest and activities in athletics conflicted with the heavy laboratory schedule of an entomology major and so he shifted to the physical education major and there earned his AB degree. Following his service in World War II, however, he returned to his first and enduring loves: the University of California and Entomology. His keen intellect, strong motivation and perseverance led Professors Ray F. Smith and E. Gorton Linsley to encourage him to work directly for his PhD, which he did under the direction of Professor Smith. As a fledgling PhD he became a member of the University of Hawaii faculty from 1949 to 1951 when he joined the University of California at

Riverside, transferring to the Berkeley campus in 1963.

Professor van den Bosch was a leading researcher and spokesman for the discipline of biological control. The practice of biological control, based primarily on taxonomy, population ecology and insect behaviour, encompasses even wider areas and includes evolution and zoogeography as well as agriculture, forestry and public health. Robert van den Bosch was knowledgeable in all these areas and solidly grounded in several of them. Often accompanied by his wife, Peggy, he searched indefatigably for natural enemies of insect pests on the continents of Europe, Africa and Asia, winning the lasting respect and deep friendship of biological control associates in every country he visited.

These world-wide explorations over the years led to successful introductions into California of natural enemies of oriental fruit fly, black scales, spotted alfalfa aphid, walnut aphid, Egyptian alfalfa weevil, and of various pests of ornamental plantings.

The detailed and scholarly work which van den Bosch carried out in these applied biological control programs contributed much basic knowledge to entomological and ecological science. His early work in Hawaii, with collaborators, on various parasites introduced to control the oriental fruit fly, included the discovery, characterisation and exploitation of a previously unknown egg-larval parasite. These Hawaiian studies provided important information on the nature of parasite competition, succession, and the theory of, and evaluation of effects of, multiple parasitism.

Another major biological control contribution concerned the spotted alfalfa aphid in California. While development of a variety of alfalfa resistant to this pest came later, the prior introduction of parasites of the aphid had a major role in the early management of this serious pest, and it still has a significant role. Van den Bosch was the key figure in these introductions—discovering and importing from the Middle East and Mediterranean areas the parasites which were ultimately responsible for success. He carefully followed the progress of the pest aphid and its natural enemy populations in the field for several years. One of his findings was that there may be a certain critical inoculation density necessary when colonising a new parasite in order to obtain successful establishment. He also obtained fundamental data relating to the success of a parasite in one climatic area but its failure in another which helps to clarify our knowledge of the patchiness of biological control successes and failures.

Foreign exploration for parasitic and predatory insects is not an easy task and, commonly, the results cannot be assessed for some years. In 1960 Professor van den Bosch collected a parasite of the walnut aphid in France and shipped it to California where it was released in Persian walnut growing areas of the state. It became established and effective, however, only in the coastal areas of southern California. In 1968 Van searched in Iran, a region climatically more similar to that of the interior and middle regions of California, for a parasite that might be better adapted. Though he found in Iran the same taxonomist's species that he had collected in France he suspected that the Iranian stock represented a different and potentially more valuable biotype. The Iranian biotype was then colonised widely in northern California, became established immediately, and by 1970 had effectively controlled the aphid.

The successful biological control of the spotted alfalfa aphid and the walnut aphid reduced crop losses and treatment costs thus saving millions of dollars to Californian agriculture.

Concurrent with and inherent in much of this 'exploration and establishment' research, Professor van den Bosch studied a number of both basic and applied problems—for example, ones relating to host specificity, timing or phenology, trophic relationships, and impact of a number of parasites on their hosts, e.g. on alfalfa weevils and several pest aphids on alfalfa, walnuts and ornamental plants. He also studied the role of hyperparasites which attack beneficial primary parasites. He made fundamental studies on the haemocytic encapsulation of weevil parasites and related this to biological control success or failure.

While Professor van den Bosch's activities as an energetic, vocal and successful researcher in biological control are widely known, his contributions to the development and theory of modern integrated pest management (=integrated control) are sometimes overlooked. In 1959 he joined with Ray F. Smith, Vernon M. Stern and K. S. Hagen to explicitly formulate the notion of the 'economic threshold' of pest impact and put forward forcefully the idea of integrating chemical, cultural and biological control tactics in a broad strategy of pest *containment*, rather than that of *preventative* or *exterminative* management by use of pesticides. This approach has now been developed further and is currently being fostered at the highest levels of government and 'implemented' increasingly in many parts of the world with, of course, varying degrees of sophistication and success.

Alfalfa and cotton in California

became van den Bosch's focal points for developing his concepts and applications of integrated control. He was among those many in California and elsewhere who contributed greatly to demonstrate not only the tremendous adverse impact on natural enemies but on the ecosystem otherwise, of pesticides when used in an arbitrary unilateral manner. In so doing, these researchers have brought to the surface the enormous amount of naturally-occurring biological control that passes unnoticed until it has been 'upset' by use of pesticides. Professor van den Bosch illustrated, along with these others, how this naturally-occurring biological control can be conserved and augmented.

Van saw the world from the dual perspective of a scientist and an alarmed layman and environmentalist. Early on, he became acutely aware of the awesome problems arising from indiscriminate use of potent, synthetic, broad-spectrum, long-lasting *biocides* in our agricultural ecosystems. This was unacceptable to him as a scientist. It threatened the discipline to which he was devoted, and it represented an assault upon invaluable resources, the biological control agents, without which the insect pests would become truly agents of disaster. It was also an assault upon our basic supporting environment itself. In recent years a major part of his energies were devoted to speaking, writing and advocating that this problem be recognised and that the necessary research, education and implementation undertaken to correct it.

Perhaps more than anyone else in the world, Professor van den Bosch, an inspiring teacher and scientist, was successful in conveying to students all over the world and to the various elements of society in general an awareness that pesticides are not simply an unmitigated blessing—that, quite the contrary, as they have been used they can be as much a threat to agricultural production as they are to human well-being and environmental integrity. This is perhaps his greatest single achievement and for which he will be remembered.

This deep concern of Professor van den Bosch's for our environment and his contempt for those who defile it were expressed in his inimitable and provocative style in his various writings and last book, *The Pesticide Conspiracy*. This book should be read by all concerned citizens of the world.

Lastly, Van loved life. His was a bright spirit and a fighting heart; perhaps it is this that his friends and colleagues shall miss the most.

Carl Huffaker
Nettie Mackey
and other friends

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